The author herby certifies that the use of any copyrighted material in this thesis manuscript entitled:

"Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents"

is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

Roberta P. Lavin
Graduate School of Nursing
Uniformed Services University of the Health Sciences

Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents

By

Captain Roberta P. Lavin

U. S. Public Health Services Lanuages

Dissertation submitted to faculty of the
Graduate School of Nursing
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Doctor of Philosophy 2008

Acknowledgements

I knew when I was twelve years old that I wanted to be a nurse. By the time I was in college my focus had moved to nursing in underserved populations and for those with the greatest needs. My journey brought me to the United States Public Health Service and to the Uniformed Services University of the Health Sciences, two institution that embody the best of nursing and service to those in need. I would like to acknowledge the many people that were critical to my success, but a few were exceptionally supportive.

There are many people at the Uniformed Services University that added to my academic experience. My committee, Drs. Christine Kasper, John Kalinich, Roopa Biswas, and Marguerite Littleton Kearney, was ever present and quick to respond. Each provided expertise and personal encouragement. There are other members of the University community that played a special role in my experience, specifically, Dr. Mark R. Adelman, TSgt Shannon Beach, LCDR Stephanie Bardack, Dr. Dorianne Watts, and LT Myovel Jean Paul.

Others who befriended me include, Carmen Contreras-Sesvold who provided guidance and assistance in many aspects of my educational process and came in over Christmas to make sure things went well. Christy Emond encouraged me, shared her office with me, and expressed genuine concern about my well being. Chaucer Grower was the comforting and supportive voice ever doctoral candidate needs. And, Ashira Lavine was always available to critique a paper and make sure my life at work was a little less demanding.

As with any journey there are those that guide us, hold our hands, and then let us choose our own path. Dr. Patricia Hinton Walker was understanding and supportive when I changed my dissertation topic. I will always regret disappointing her and appreciate her support even when she though I might be making a mistake.

Finally, I would like to acknowledge and dedicate my dissertation to my husband Michael Lavin. He has been a constant source of encouragement and support for the last 22 years. He is always ready with the perfect book, calming words, and the wisdom to know when it is time to take a break. Most importantly, he believes in the quest for knowledge and taught me that gaining knowledge is a necessary step to creating new knowledge and sharing it.

Table of Contents

Lavin, R., Dreyfuss, M., Slepski, L., & Kasper, C. (2007) Subject Matter Experts: Fact or Fiction. *Nursing Forum, 42(4).*

Lavin, R., Harrington, M., Lavine, A., Agbot-Tabi, E. & Erger, N. (2007) Critical Infrastructure Protection: Why Physicians, Nurses, and Other Healthcare Professional Need to be Involved. *American Journal of Disaster Medicine,* 1(1), p 48 – 54.

Savitz, L., **Lavin, R**. & Root, E. (2007). Geographical Information Systems as a Strategic Tool for Better Planning, Response, and Recovery. In *National Security Issues in Science, Law, and Technology.* Taylor & Francis, Boca Raton.

Lavin, R., Slepski, L., & Veenema, T. (2007) Leadership and Coordination in Disaster Health Care Systems: The Federal Disaster Response Network. In Veenema, T. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.

Riccardi, R., Agazio, J., **Lavin, R**., & Walker, P. (2007) Directions in Nursing Research and Development. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.

Lavin, R. & Dreyfuss, M. (2007) HIPAA Case Study. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards,* 2nd Ed., New York, Springer Publishing Co.

Reeves, G., Lavin, R**.,** & Slepski, L. (2006) *Online Preparedness Education Program. Radiation Dispersal Device.* Available at http://opep.usuhs.edu/overview_acknow.do**.**

Lavin, R. (2006) HIPAA and Disaster Research: Preparing to Conduct Research. *Disaster Management and Response,* 4(2), 32-37.

Chaffee, M., **Lavin, R.** & Slepski, L. (2006)Nursing Role: Homeland Security. *Current Issues in Nursing*, 7th Ed. Mosby, St. Louis, MO.

Couig, M., Martinelli, A., & **Lavin, R.** (2005) The National Response Plan: Health and Human Services the Lead for Emergency Support Function #8. *Disaster Management & Response,* 3(2), 34-40.

Phillips, S. & **Lavin, R.** (2004) Readiness and Response to Public Health Emergencies. *Journal of Professional Nursing.* November, 2004.

E: Complete Bibliography & Additional Table of Work while in Doctoral Program

Executive Summary

It has been estimated that over 28,451 Americans have been wounded in Iraq since March 2003. Fragmentation wounds accounted for approximately forty-nine percent of wounds in the Persian Gulf War and forty-six percent of wounds in the first phase of Operation Iraqi Freedom. For most, the shrapnel will have caused its damage at the time of the injury and the soldier will suffer no further harm. However, in addition to causing skeletal muscle pathology, munitions composed of heavy metals, such as depleted uranium and the tungsten alloys, also have chemical properties that are potentially carcinogenic.

The expanded use of heavy metals in munitions and the introduction of new materials, such as explosively-shaped charges, on the battlefield increase the risk of shrapnel wounds in both combatants and noncombatants. Knowledge of the long-term consequences of gunshot and shrapnel injuries is now an issue for both military and civilian health care and timely and proper care is critical for those wounded. In particular, the recent report that a military-grade tungsten alloy induced aggressive rhabdomyosarcomas (RMS) when implanted in the leg muscles of laboratory rats raises serious questions as to the timing of skeletal muscle changes that lead to neoplastic transformation.

Therefore, the primary aims of this study are: 1) to characterize the ultrastructural and morphologic forms of skeletal muscle damage characteristic of the presence of embedded WA, 2) to determine if the rate and magnitude of the development of early signs of damage, or if changes in the ultrastructure and morphology of skeletal muscle,

are early neoplastic indicators, and 3) to determine the rate and magnitude of the development of RMS.

In the second manuscript, the potential health effects of tungsten alloy are addressed. In manuscript three, the importance of understanding the current state of the science of retained fragments is clarified, as all nurses—and indeed most Americans—are concerned about the care of victims of violence whether it occurs in Iraq or on our city streets. Many victims of violence with injuries involving retained fragments leave the military before long-term complications become apparent. In fact, while there are a few articles on wound ballistics and immediate care of persons with gunshot and shrapnel wounds in the nursing literature, they do not address the debate over removal of embedded fragments, the likely long-term implications, or the differences between civilian and military injuries. The manuscript concludes with a clarification of the critical role nurses must play in policy formulation related to the long-term care of victims of violence. Manuscripts four and five address the specific research methods and findings, which follow in brief.

Four groups of Fischer 344 male rats were used for the pellet implantation study. A negative control group was implanted in the gastrocnemius muscle with tantalum (Ta) pellets, a positive control group was implanted with nickel (Ni) pellets, and two experimental groups were implanted with either a high dose (20 pellets) or low dose (4 pellets) of WA pellets. To keep metal loads identical, those in the low-dose WA group also received 16 Ta pellets, thus every rat was implanted with 20 pellets. The number and dimensions of the pellets (cylinders, 1 mm in diameter x 2 mm in length) were based on research previously conducted at the Armed Forces Radiobiology Research

Institute. The WA pellets consisted of 91.1% tungsten, 6% nickel, and 2.9% cobalt, similar to one of the tungsten alloys used in kinetic-energy penetrators. Tantalum was chosen as the implantation control metal because it is biologically inert with a mass similar to tungsten. Nickel was used a positive control because it is a known carcinogen. Rats were euthanized at 1, 3 and 6 months and muscle and tumor samples collected.

No tumors developed in the Ta-implanted animals and no indications of neoplastic changes were seen around the implanted pellets. 100% of the Ni-implanted rats developed large tumors by 6 months as did both the low- and high-dose-implanted rats. Tumor development was slower in the WA low-dose group. Implantation with WA pellets also resulted in increased vascularity, high mitotic cell number, and both apoptosis and necrosis of the muscle.

The concluding portion of the study used multiple histochemical stains to identify changes in muscle that occur prior to the development of RMS in rats implanted with WA. The four major findings from this section are: 1) severe atrophy as early as 1-month post implantation, 2) neoplastic changes as early as 1-month post implantation, 3) a marked destruction of myofibers starting at 1-month post implantation, and 4) an increase in collagen spreading outward from the pellet implantation site as early as 1-month post implantation.

This study, as with those previously published, raises concern about the potential health effects, including carcinogenicity, of the tungsten alloys. Based on recently published data, the greatest concern may be with embedded fragments of WA suffered as the result of a shrapnel wound. Standard surgical guidelines recommend leaving

fragments in place. This study shows that even at low dose exposures this may not be a wise decision with embedded WA. This study, while not definitive, suggests that regular monitoring of those with shrapnel injuries containing heavy metals may be warranted.

Nurses must carefully monitor patients with these injuries and be aware of the health issues that embedded fragments present. In addition, it is time to reexamine the topic of fragment removal and educate healthcare professionals on the potential long-term health consequences of embedded fragments, develop longitudinal studies that follow those persons with embedded fragments, and explore policy options that ensure that victims of violence are not injured twice.

HIPAA and Disaster Research: Preparing to Conduct Research

Roberta P. Lavin, MSN, APRN-BC

The Health Insurance Portability and Accountability Act (HIPAA), enacted in 1996 and implemented in 2003, continues to have a profound impact on the ability to carry out research. Many disaster research methodologies are not affected by the HIPAA Privacy Rule and are likely to be appropriate for waivers because of the nature of disaster research. Still other types of studies may require special considerations that researchers must be aware of when planning research methodologies.

The Health Insurance Portability and Accountability Act (HIPAA) was enacted by the United States Congress to address 2 goals: (1) the need for insurance portability when individuals change or lose a job, and (2) the need to protect individuals from potential threats to their privacy posed by electronic health information. After public review, the Department of Health and Human Services posted the final rule in 2002, and it went into effect in April 2003. The Privacy Rule, a subsection of HIPAA, has resulted in sweeping changes in the way health care providers, organizations, and clearinghouses are required to maintain and disclose health information. Among the most notable changes were the requirements to inform patients of their right to review their health record for accuracy and to obtain their written approval before sharing protected health information (PHI) with anyone who does not have a legitimate reason to see it.

Disaster response efforts rely on many health professionals, especially nurses. During a disaster response, states and hospitals request for more nurses more than any other health care professionals. This high demand has led nurse researchers to address the critical issues that affect nurses during a disaster response. However, it is not clear what impact the HIPAA Privacy Rule has on research in general. Some investigators are concerned that the HIPAA guidelines might slow research,[1,2] that the need to de-identify data will result in less valuable findings,[3] and that complying with the Privacy Rule will require an excessive amount of time and cost. Still others claim that the issue should not be whether research can be done without impediment but whether individual privacy is protected.[4]

It is not clear what impact the HIPAA Privacy Rule has on research in general.

This article has 2 specific purposes: (1) to identify the realities of the HIPAA Privacy Rule and how it applies to disaster research, and (2) to provide guidelines to help researchers navigate through the Privacy Rule requirements when designing research. The goal is to explain the HIPAA Privacy Rule and help researchers comply in order to promote the quantity and diversity of disaster-related research.

The HIPAA Privacy Rule and How It Applies to Disaster Research

The HIPAA Privacy Rule has been in effect for approximately 3 years and increasingly has become viewed as a roadblock to research. The rule was established to protect individually identifiable health information from unauthorized disclosure, use, or access by a covered entity[5] and was not intended to impede new knowledge. It is important that researchers understand key elements of the HIPAA Privacy Rule in order to be able to conduct research and comply with the rule. Key elements to review are "covered entities," "valid authorization," and "public health exceptions."

Covered Entities

The HIPAA Privacy Rule defines covered entities as hospitals, health plans, health care clearinghouses, and health care providers who electronically transmit PHI.[5] HIPAA also states that not all organizations that

Roberta P. Lavin is a Doctoral Student, Uniformed Services University for the Health Sciences, Bethesda, MD.

Reprint requests: Roberta P. Lavin, MSN, APRN-BC, 1221 S Eads St, #1411, Arlington, VA 22202; E-mail: rlavin10@mac.com

Disaster Manage Response 2006;4:32-37.

1540-2487/$32.00

doi:10.1016/j.dmr.2006.01.003

deal with PHI are considered covered entities. Public health departments that do not provide direct patient care (examples of what are considered direct patient care include human immunodeficiency virus [HIV] screening and childhood vaccinations); agencies that work with worker compensation programs; and all employers, some insurers, and those that provide social welfare services are not considered covered entities, and the information they collect is not subject to the Privacy Rule. Olsen[6] warns that most nursing research will be covered under the Privacy Rule because of the nature of nursing research and the fact that most nurses work in or are associated with covered entities.

Valid Authorization

The HIPAA Privacy Rule states that if *patient information from a covered entity* is used in research, authorization must be obtained from each individual involved in the study before that health care information can be used. This rule applies even if the researcher does not work for the covered entity. The specific policies that involve obtaining valid authorization for the use of PHI are listed in Table 1.

Table 1. Policies that affect the valid authorization of the use of Protected Health Information (PHI)

1. A written authorization must have a specific description of the information that will be used.
2. The name of the persons or group of persons using the PHI must be listed.
3. The persons who will disclose the PHI are listed.
4. The authorization has an expiration date.
5. A clearly written statement must be included that states that the authorization may be revoked at any time upon the request of the individual.
6. The authorization must be signed by the individual or an authorized representative.

(Health Insurance Portability and Accountability Act of 1996, 2000b.)

Additionally, if the request for authorization comes from the covered entity, then the covered entity is responsible for ensuring that the individual understands the following:

1. Why the information is being requested and for what purpose it will be used;
2. That he or she may review the information before it is released and refuse to release the information;
3. That authorization to release PHI is not a requirement for treatment, payment, enrollment, or eligibility for care; and
4. Whether financial compensation is being provided to the covered entity.[8] The Privacy Rule specifies that this must be written and not merely verbal.

Exemptions to Privacy Rule

Minimum necessary. Agencies are allowed to disclose individual information "…related to law enforcement, judicial proceedings, national security, familial contact, minors, health research, and notably, public health."[7] However, even with this exemption, the Privacy Rule only allows for the disclosure of the *minimum necessary* PHI to accomplish the purpose. Researchers must carefully consider and document how the decision was made to determine the "minimum necessary." Most institutions have policies covering these issues, and all institutions are required to have a designated individual to whom questions can be addressed.

Public health responsibilities. PHI can be released without written authorization by a covered entity to public health agencies if required to do so by law. Public health activities that are exempted include disease control and surveillance, intervention efforts, investigating exposure to infectious diseases, some types of workplace surveillance, and information needed to avert serious threats to health or safety. Covered entities are still required to document the release of any PHI because the individuals whose PHI is involved have a right to know to whom it was released, even though it was legal to release it without consent.

PHI can be released without written authorization by a covered entity to public health agencies if required to do so by law.

Exceptions for research. Covered entities may not be required to obtain written authorization if the PHI is to be used for research, "…provided that an Institutional Review Board (IRB) or privacy board … approves the waiver of individual authorization required under §164.508 of the regulation and certain other conditions are met."[8] Researchers may use limited data sets and de-identified data, thereby posing no significant risk to privacy as long as protocols are in place to ensure the security of PHI. (IRB waivers are discussed in greater detail under "Complying with HIPAA and Preparing for Research," in this article.)*

How the Privacy Rule Affects Disaster-Related Research

The Privacy Rule may apply in some research situations but not in others, depending on the study's

*The HIPAA Privacy Rule allows reviews by IRBs and privacy boards. For the purpose of this article, IRB will be used for ease of reading.

design. Research is defined by the Privacy Rule and the Common Rule for the Protection of Human Subjects[9] as "systematic investigation—including research development, testing, and evaluation—designed to develop or contribute to generalizable knowledge."[7]

Exemptions. Studies that do not involve PHI (e.g., observational studies of prehospital social behavior, research on just-in-time educational training for health care professionals, and modeling of patient flow) are considered exempt from compliance with the Privacy Rule. Public health activities such as prevention, disease surveillance, and reporting usually are exempt because they are considered essential public health functions and not research. Disease registries maintained by public health agencies are not covered under the Privacy Rule.

Indications. Many researchers argue that written authorizations are inappropriate for disaster research and it is impractical to get authorizations after a mass casualty event. Currently, if the research uses PHI obtained during a disaster, the researchers will need authorization. Because disasters are unpredictable, it will be essential for the investigators to obtain Privacy Rule waivers or have an IRB pre-approved authorization form prior to collecting PHI data during a disaster. If a public health agency or a person performing a public health function designs a research study that is meant to contribute to generalizable knowledge, then it is considered research and becomes subject to the Privacy Rule.

Case by case decisions. The Centers for Disease Control and Prevention (CDC) has interpreted a distinction between practice and research interpretation within the Privacy Rule. They define the collection of epidemiologic information for the purpose of tracking an outbreak to not be research, even though it may be reported in the literature and contribute to generalizable benefits. The CDC also notes that activities that start as public health activities eventually may become research activities. For example, if disease outbreak tracking is linked to a clinical drug trial, then it is considered to be research. In this case, researchers must follow the rules for research but should not stop data collection during the outbreak because they are going through the Privacy Rule process.[10] Additional resources on public health and the Privacy Rule can be accessed at *www.cdc.gov/mmwr*.

In some situations, public health agencies may use limited data sets (i.e., city, zip codes, date of birth or death, and discharge dates) provided that a covered entity has a data-use agreement with the recipient of the information. This agreement must specify who will use the PHI and that the PHI will not be disclosed further, will not be re-identified, and that any misuse will be reported to the covered entity.[10]

Guidelines to Navigate the Privacy Rules for Disaster-Related Research

Designing Research Studies

Nonexperimental research. In nonexperimental studies, the investigator does not manipulate or control factors, but instead describes (i.e., descriptive), compares (i.e., comparative) or demonstrates correlations of phenomena (i.e., correlational). During the past decade the majority of disaster studies have been descriptive.[11] Whether these studies require compliance with the Privacy Rule depends more on the setting and data collected. For example, if a study is of nurse behavior within a covered entity, then the rule does not apply; if it looks at patient behavior and utilizes any PHI, the Privacy Rule does apply.

Quasi-experimental designs. Quasi-experimental designs are defined as studies that do not use random assignment of subjects. This design is being used more in disaster research and is useful for evaluating outcomes, such as the return of whatever is being studied to normal after a disaster. For example, casualty triage is based on established criteria. During a mass casualty event, a quasi-experimental study might explore the point at which saturation of triage resources occurs and at what point it returns to normal. Quasi-experimental studies are not likely to involve Privacy Rule considerations unless the evaluation of triage is linked to PHI.

Data mining. As large data sets residing on institutional servers become available to researchers, data mining for research purposes is growing.[12] When using data, it is important to consult the local IRB for assistance in working through the process, especially in determining whether the database is part of a covered entity. Data sets can be part of a covered entity or part of public health epidemiologic data.

Although many data sets may pre-date the Privacy Rule, researchers must still comply with the Privacy Rule when using mined data. Researchers using older data sets are required to have written authorizations, de-identify the data, or obtain a waiver. Some persons have argued that if there is a "silver lining" to the Privacy Rule, it is the improvement in data transfer technology that is resulting in better data sets.[13] Others claim that due to the expense and time requirements of complying with HIPAA, valuable data sets will be lost and population-based studies will no longer be population based because written authorization may result in sample

Although many data sets may pre-date the Privacy Rule, researchers must still comply with the Privacy Rule when using mined data.

bias.[14] It will be difficult, if not impossible, to track down the individuals in the existing data sets.

Complying with HIPAA and Preparing for Research

Essential steps that investigators can take in advance to comply with the Privacy Rule include (1) accessing data from a covered entity, (2) creating data, and (3) "disclosing data."[6] Researchers conducting disaster research most likely will need to seek a waiver for authorization if the research is to be done at the time of an actual disaster.

Prepare for research. Researchers can obtain some information, including PHI, from a covered entity in preparation for research. The researcher must assure the covered entity: (1) that the review is only to "prepare a research protocol," (2) that no PHI will be removed from the covered entity, and (3) that "the PHI is necessary to plan the research."[15] This can be done with the consent of the covered entity and without an IRB approval. For disaster researchers who may be doing research during a disaster, this process helps the researcher to become familiar with staff and operations.

Obtain valid authorization to disclose PHI. The Privacy Rule requires that a written authorization be given prior to accessing PHI. The authorization form must be complete, specific, and in plain language. The local covered entity already may have the appropriate forms that only require the specific information to be inserted. Once signed, the researcher must give the individual a copy of the signed authorization form. Though the authorization can be waived, IRBs are reluctant to do so.[16]

IRB waivers. The Privacy Rule allows researchers to obtain a waiver from their local IRB to collect PHI without a written authorization, provided that the researcher implements special handling of PHI.

1. Minimal risk: Section 164.512(i) states that if research involves no more than a minimal risk to privacy and the research could not practically be carried out without it, then the local IRB may grant a waiver provided steps are taken to protect PHI. The technical requirements can be found in part 160 and 164 of the HIPAA.[17]
2. Statistical probability: The most useful method for justifying minimal risk and the need for the PHI is the statistical probability method as cited in 45 CFR § 164.514(b), which requires that "a properly qualified statistician using accepted analytic techniques concludes the risk is substantially limited that the information might be used, alone or in combination with other reasonably available information, to identify the subject of the information."[10] In this method all information must have 128-bit encryption prior to being uploaded to a server. It also must ensure that the information can be de-identified and then re-identified. Kline et al[17] provide an excellent example of this process.
3. Safe harbor: Alternatively, the safe harbor method sited in 45 CFR § 164.514(b) allows the IRB to grant a waiver if a "covered entity or its business associate de-identifies information by removing 18 identifiers (see Table 1) and the covered entity does not have actual knowledge that the remaining information can be used alone or in combination with other data to identify the subject."[10] This can be problematic if for any reason follow-up would become necessary.

Finally, even if a waiver is granted, it is still necessary for the covered entity to maintain records of the release of any PHI for 6 years because patients have a right to know to whom their information was released. Large hospitals and research institutions likely will have systems in place to document the release of PHI. There is little doubt that signed authorization is a barrier to research. However, security procedures are not implemented to increase convenience—they are implemented to protect, and in this case they protect the privacy of patients. This does not mean that research must stop; it means that prior planning is essential.

There is little doubt that signed authorization is a barrier to research.

PHI of deceased persons. Researchers who include records of deceased persons are required to prove that the information is vital to the study and that the person is indeed deceased. These requirements vary from state to state, so the researcher will need to check each state's laws. This process can become time-consuming and costly if a large number of records of deceased persons are being reviewed.

Minimum necessary. The term "minimum necessary" is not well defined, and each IRB will need to review every research protocol to determine that the PHI being released is the "minimum necessary." The IRB must be provided adequate documentation to justify the extent to which release of PHI is necessary. It is unlikely that organizations will accept the review of another IRB because it does not remove their liability. Researchers should plan on multiple IRB reviews if data collection will occur at multiple locations.

Use of partial data. Both limited data sets and de-identified data can be used for research without authorization. The 18 data elements that cannot be released without authorization are listed in Table 2. It has been suggested that the process of de-identification can produce data that is less complete and much

Table 2. Subject identifying data elements that cannot be released without prior authorization

1. Name
2. Geographic divisions smaller than a state, except for first 3 digits of the zip code
3. Dates of birth, death, admission, or ages greater than 89 years
4. Driver's license or car license numbers
5. Social security numbers
6. Medical record numbers
7. Health plan numbers
8. Account numbers
9. Phone numbers
10. Fax numbers
11. E-mail addresses
12. License numbers
13. Any vehicle identification numbers
14. Any medical device or serial numbers
15. Internet URLs
16. Internet IP addresses
17. Biometrics
18. Any other unique identifier that allows de-identified information to be re-identified

Adapted from 45 CFR § 165.514(b)(2)(i).

less useful.[3] If a limited data set retains some identifiers, including "age, date of birth and death, zip code (5 digits only), state, county, city, geocode, dates of admission and discharge, and other characteristics or codes not listed as direct identifiers,"[15] the users must have a data use agreement with the covered entity that complies with the Privacy Rule. Data that is truly de-identified of PHI and confirmed by a statistician is less useful for health services research because it removes too much information to make the research useful.[18]

Determining which law rules. If there is disagreement between a state and Federal law, the most restrictive law, or the one that provides for the greater privacy for the patient, will apply. The Privacy Rule will predominate in states where state law for the protection of PHI is less restrictive than the Federal law.

If there is disagreement between a state and Federal law, the most restrictive law, or the one that provides for the greater privacy for the patient, will apply.

Creating and disclosing data. Research, especially intervention studies, can result in the creation of PHI. If these studies are done within a covered entity, then the covered entity should provide the individual with a Notice of Privacy Protection. If the research is conducted outside of a covered entity, then the researcher is responsible for ensuring that notice is provided and consent is obtained from the individual. Intervention studies are unlikely during disaster research; however, surveys and interviews are likely to be used in disasters. These usually do not result in the creation of PHI and are not subject to HIPAA if no PHI is created. Once PHI is created, if the researcher shares the information with others, it is considered disclosure under HIPAA.[6] It is best to simplify the process by making sure that anyone who is going to have access to the PHI is listed on the authorization form or covered in the IRB waiver.

Conclusion

The HIPAA Privacy Rule is a legal matter, and researchers should not allow it to slow the progress of advancing knowledge. Although it appears that HIPAA has adversely affected health services research and affected research topics and methodology, preparedness for research can help smooth the road to IRB approval. Researchers encounter problems with HIPAA when they involve actual patients. Research methodology should not be selected to avoid the need for IRB approval or other inconveniences, such as excessive time, the fear of denial, and costs.

HIPAA has been described as one of the worst things that ever happened to science, and IRBs and policy makers must ask whether the risk to privacy outweighs the risk to lost research. In the meantime, those who care about and work in disaster response must remember that the purpose of research is to benefit people. Even though the cost of doing research is increasing, researchers must learn how to navigate the system and work within it to serve patients.

References

1. Coy KL. The current privacy environment: implications for third-party research. J Contin Educ Health Prof 2001; 21:203-14.
2. Vates JR, Hetrick JL, Lavin KL, Sharma GK, Wagner RL, Johnson JT. Protecting medical record information: start your research registries today. Laryngoscope 2005;115: 441-4.
3. Clause SL, Triller DM, Bornhorst CP, Hamilton RA, Cosler LE. Conforming to HIPAA regulations and compilation of research data. Am J Health Syst Pharm 2004;61:1025-31.
4. Annas GJ. Medical privacy and medical research—judging the new federal regulations. N Engl J Med 2002; 346:216-20.
5. Health Insurance Portability and Accountability Act of 1996. Pub. L. No. 104-191, 110 Stat. 1936 (1996).
6. Olsen DP. HIPAA privacy regulations and nursing research. Nurs Res 2003;52:344-8.
7. Kamoie B, Hodge JG Jr. HIPAA's implications for public health policy and practice: guidance from the CDC. Public Health Rep 2004;119:216-9.
8. Borzi PC. Protecting individual medical records: a description of key requirements of the final regulations applicable to group health plans. Washington: O'Donoghue & O'Donoghue.

9. Protection of Human Subjects. CFR 45 Part 46.102(d), 1999.
10. Centers for Disease Control and Prevention. HIPAA privacy rule and public health. Guidance from CDC and the U.S. Department of Health and Human Services. MMWR Morb Mortal Wkly Rep 2003;52(Suppl):1-17, 19-20.
11. Health disaster management guidelines for evaluation and research in the Utstein style. In: Sundenes KO, Birnbaum ML, Birnbaum ED, editors. Madison, WI: Prehospital and Disaster Medicine; Vol. 17, 2003.
12. Windle PE. Data mining: an excellent research tool. J Perianesth Nurs 2004;19:355-6.
13. Durham ML. How research will adapt to HIPAA: a view from within the healthcare delivery system. Am J Law Med 2002;28:491-502.
14. Kaiser J. Patient records. Privacy rule creates bottleneck for U.S. biomedical researchers. Science 2004;305:168-9.
15. Gunn PP, Fremont AM, Bottrell M, Shugarman LR, Galegher J, Bikson T. The Health Insurance Portability and Accountability Act Privacy Rule: a practical guide for researchers. Med Care 2004;42:321-7.
16. Ingelfinger JR, Drazen JM. Registry research and medical privacy. N Engl J Med 2004;350:1452-3.
17. Kline JA, Johnson CL, Webb WB, Runyon MS. Prospective study of clinician-entered research data in the Emergency Department using an Internet-based system after the HIPAA Privacy Rule. BMC Med Inform Decis Mak 2004;4:17.
18. Kulynych J, Korn D. The effect of the new federal medical-privacy rule on research. N Engl J Med 2002;346: 201-4.

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — **New Investigator** ☐ **No** ☒ **Yes**

3a. NAME (Last, first, middle)
Lavin, Roberta, Proffitt

3b. DEGREE(S)
BS MSN MA

3h. eRA Commons User Name

3c. POSITION TITLE
PhD Student

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Uniformed School University of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814-4799

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Uniformed Services University of the Health Sciences

3f. MAJOR SUBDIVISION
Graduate School of Nursing

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 202-205-4782 FAX: 202-690-6512

E-MAIL ADDRESS:
roberta.lavin@hhs.gov

4. HUMAN SUBJECTS RESEARCH	4b. Human Subjects Assurance No.		5. VERTEBRATE ANIMALS ☐ No ☒ Yes	
☒ No ☐ Yes	4c. Clinical Trial ☒ No ☐ Yes	4d. NIH-defined Phase III Clinical Trial ☒ No ☐ Yes	5a. If "Yes," IACUC approval Date	5b. Animal welfare assurance no.
4a. Research Exempt ☒ No ☐ Yes	If "Yes," Exemption No.		Not required - preexisting samples	A3448-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
11/01/06	11/01/07				

9. APPLICANT ORGANIZATION
Name: The Henry Jackson Foundation
Address: Advancement of Military Medicine, Inc.
1401 Rockville Pike, Suite 600 Rockville, MD 20852

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1521317896A1
DUNS NO. 14-467-1276 Cong. District 8th MD

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Craig D. Anderson
Title: Chief Financial Officer
Address: The Henry Jackson Foundation
Advancement of Military Medicine, Inc.
1401 Rockville Pike, Suite 600 Rockville, MD 20852
Tel: 301-294-1276 FAX: 301-294-1292
E-Mail: ospnga@hjf.org

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Sudha Sringagesh
Title: Senior Grants Manager
Address: The Henry Jackson Foundation
Advancement of Military Medicine, Inc.
1401 Rockville Pike, Suite 600 Rockville, MD 20852
Tel: 301-294-1276 FAX: 301-294-1292
E-Mail: ospnga@hjf.org

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* DATE

Use only if responding to a Multiple PI pilot initiative. See http://grants.nih.gov/grants/multi_pi/index.htm for details.

Contact Principal Investigator/Program Director (Last, First, Middle):		
3. PRINCIPAL INVESTIGATOR		
3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*	
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT		
3f. MAJOR SUBDIVISION		
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:	
3. PRINCIPAL INVESTIGATOR		
3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*	
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT		
3f. MAJOR SUBDIVISION		
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:	
3. PRINCIPAL INVESTIGATOR		
3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*	
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT		
3f. MAJOR SUBDIVISION		
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:	
3. PRINCIPAL INVESTIGATOR		
3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. NIH Commons User Name
3c. POSITION TITLE	3d. MAILING ADDRESS *(Street, city, state, zip code)*	
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT		
3f. MAJOR SUBDIVISION		
3g. TELEPHONE AND FAX *(Area code, number and extension)* TEL: FAX:	E-MAIL ADDRESS:	

DESCRIPTION: See instructions. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project (i.e., relevance to the **mission of the agency**). Describe concisely the research design and methods for achieving these goals. Describe the rationale and techniques you will use to pursue these goals.

In addition, in two or three sentences, describe in plain, lay language the relevance of this research to **public** health. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The long-term goal of in situ shrapnel identification is to give the patient treatment options that will result in the best outcomes. Each person will differ in existing health status, degree of shrapnel injury, and ability to return to optimal health. There is evidence that even shrapnel injuries not involving heavy-metal tungsten-alloy (HMTA) can result in tumor formation and other long-term health consequences. The indicators of these long-term adverse health effects have not been identified. The overall objective is that data from this research study will provide indications of early signs of skeletal muscle changes that may indicate impending adverse consequences of embedded HMTA. The central hypothesis of this project is that there is a significant difference between the magnitude of skeletal muscle damage in Fisher 344 rats embedded with HTMA and those embedded with either positive (nickle) or negative (tantalum) controls.

The specific aims are as follows: 1) To characterize the ultrastructural and morphologic forms of skeletal muscle damage which are characteristic of the presence of embedded HTMA; a) Muscle damage will be measured by disintegration of muscle fiber, loss of connective tissue, change in vascularity, change in fiber type, change in area occupied by fibers per mm2, circularity of myofibers, and change in number of fibers per mm2 as compared to controls; 2) To determine if the rate and magnitude of the development of early signs of damage or change in the ultrastructure and morphology of skeletal muscle is a precancerous indicator; and 3) The rate and magnitude of the development of rhabdomyosarcoma at 3 time points (1, 3 & 6 months) will be correlated to the altered morphology and ultrastructure of the experimental muscle.

The "Carcinogenicity and Immunotoxicity of Embedded Depleted Uranium and Heavy-Metal Tungsten Alloy in Rats" research previously done will serve as the source of the histology samples, therefore the number and dimensions of the pellets of HMTA, Ni, and tatalum and the time points are based on their research. Histologic samples will be taken from each experimental group (Aim 1) and each time point (Aim 2). The rate and magnitude of the development of rhabdomyosarcoma will the be correlated to altered morphologgy of the muscle (Aim 3). The relevancy to public health is the expectation that the U.S. citizens will face shrapnel injuries from such weapons in conflicts and will need to determine the best treatment options.

PERFORMANCE SITE(S) (organization, city, state)

Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, MD 20889-5603

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator(s). List all other key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Lavin, Roberta, Proffitt		USUHS	Principal Investigator
Biswas, Roopa		USUHS	Co-investigator
Kalinich, John		AFRRI	Co-investigator
Kasper, Christine		USUHS	Dissertation Chair
Kearney, Marguerite	mkearne2	Johns Hopkins Univ	Dissertation Member
McClain, David		AFRRI	Co-investigator

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project

Human Embryonic Stem Cells ☒ **No** ☐ **Yes**

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

Number of publications and manuscripts accepted for publication *(not to exceed 10)* 0

Other items (list):

Principal Investigator/Program Director (Last, First, Middle): Lavin, Roberta Proffitt

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 11/01/06 THROUGH 11/01/07

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Lavin, Rberta, Proffitt	Principal Investigator	12				0	0	0
Biswall, Roopa	Co-investigator	12				0	0	0
Kasper, Christine	Dissertation Chair	12				0	0	0
SUBTOTALS →								

CONSULTANT COSTS Dorianne Watts		0
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		0
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)*		0

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$**
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$**

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		0	0	0	0	0
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES						
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	0	0	0	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*						
CONSORTIUM/ CONTRACTUAL COSTS	F&A					
TOTAL DIRECT COSTS						

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

BUDGET JUSTIFICATION PAGE MODULAR RESEARCH GRANT APPLICATION						
	Initial Period	**2nd**	**3rd**	**4th**	**5 th**	**Sum Total (For Entire Project Period)**
DC less Consortium F&A	*(Item 7a, Face Page)*					*(Item 8a, Face Page)*
Consortium F&A						
Total Direct Costs						$

Personnel

Consortium

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Research will be performed at AFRRI and USUHS. The USUHS Graduate School of Nursing laboratory space assigned is located in Building C, Room C1058 and is approximately 740 sq. ft. The laboratory is accredited and is equiped by the Graduate School of Nursing. The laboratory has been cleared by EHS and Radiation Safety at USUHS. See continuation page.

Clinical:

NA

Animal:

The AFRRI animal care and use program where the original study was performed is fully accrediated by AAALAC and overseen by the AFRRI Institutional Animal Care and Use Committee. No test on live vertibrate animals will be conducted in this study.

Computer:

Through USUHS multiple PC and Mac computers are available and equiped with the statistical package SPSS, graphics software, database and word processing, Internet access, and ImageJ. See continuation page.

Office:

The principal investigator has a dedicated office at 200 Independence Ave, SW and may also use the USUHS common office space that is dedicated to students including the Learning Resource Center (LRC).

Other:

The LRC is available to the principal investigator and all USUHS and AFRII staff. The LRC is contains a full variety of necessary reference materials, is assessable through the Internet, and is professionally staffed. USUHS has a complete graphics arts department.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The following equipment is currently available in the USUHS laboratory: Zeiss LSM 410 confocal microscope, Nikon Optiphot fluorescence microscope, Zeiss and Nikon surgical dissecting microscopes (2), -70C freezers (2), walk-in coldroom, Beckman Avanti J25i refrigerated centrifuge, Eppindorf microcentrifuge, BioRad and Hoeffer gel/blot electrophoresis systems, polytron, autoclave, Beckman LS2800 Scintillation counter, Perkin-Elmer Lambda 20 dual beam spectrophotometer Hewlet-Packard 8 channel physiograph, Cambridge 400a force transducers (3), Barnsted RoPure and NanoPure water purification systems, chemical fume hoods, tissue culture hoods, and convection incubator.

Through AFRRI there is a tissue culture facility; ThermoElemental PQ ExCell ICP-MS System with Cetac ASX500 Autosampler, Olympus AHBT3 photomicroscope with Nomarski differential interference-contrast and reflected light fluorescence attachments for photomicroscopy work; and Coulter Counter Model ZM with Multisizer II attachment for cell counting and sizing.

Principal Investigator/Program Director (last, First, Middle): Lavin, Roberta, Proffitt

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ REVISION/RESUBMISSION of application number: _____

(This application replaces a prior unfunded version of a new, competing continuation/renewal, or supplemental/revision application.)

☐ COMPETING CONTINUATION/RENEWAL of grant number: _____

(This application is to extend a funded grant beyond its current project period.)

INVENTIONS AND PATENTS *(Competing continuation/renewal appl. only)*

☒ No ☐ Previously reported

☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT/REVISION to grant number: _____

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

Name of former principal investigator/program director: _____

☐ CHANGE of Grantee Institution. Name of former institution: _____

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

1. PROGRAM INCOME ***(See instructions.)***

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***

In signing the application Face Page, the authorized organizational representative agrees to comply with the following policies, assurances and/or certifications when applicable. Descriptions of individual assurances/certifications are provided in Part III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

•Human Subjects Research •Research Using Human Embryonic Stem Cells •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy •Vertebrate Animals• •Debarment and Suspension •Drug- Free Workplace *(applicable to new [Type 1] or revised/resubmission [Type 1] applications only)* •Lobbying •Non-Delinquency on Federal Debt •Research Misconduct •Civil Rights (Form HHS 441 or HHS 690) •Handicapped Individuals (Form HHS 641 or HHS 690) •Sex Discrimination (Form HHS 639-A or HHS 690) •Age Discrimination (Form HHS 680 or HHS 690) •Recombinant DNA Research, Including Human Gene Transfer Research •Financial Conflict of Interest •Smoke Free Workplace •Prohibited Research •Select Agent Research •PI Assurance

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: _____ ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	_____	x Rate applied	_____	% = F&A costs	$	_____
b. 02 year	Amount of base $	_____	x Rate applied	_____	% = F&A costs	$	_____
c. 03 year	Amount of base $	_____	x Rate applied	_____	% = F&A costs	$	_____
d. 04 year	Amount of base $	_____	x Rate applied	_____	% = F&A costs	$	_____
e. 05 year	Amount of base $	_____	x Rate applied	_____	% = F&A costs	$	_____
					TOTAL F&A Costs	$	

*Check appropriate box(es):

☐ Salary and wages base ☐ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

Principal Investigator/Program Director (Last, First, Middle): Lavin, Roberta, Proffitt

Place this form at the end of the signed original copy of the application.
Do not duplicate.

PERSONAL DATA ON PRINCIPAL INVESTIGATOR(S)/PROGRAM DIRECTOR(S)

The Public Health Service has a continuing commitment to monitor the operation of its review and award processes to detect—and deal appropriately with—any instances of real or apparent inequities with respect to age, sex, race, or ethnicity of the proposed principal investigator(s)/program director(s).

To provide the PHS with the information it needs for this important task, complete the form below and attach it to the signed original of the application after the Checklist. When multiple PIs/PDs are proposed, complete a form for each. **Do not attach copies of this form to the duplicated copies of the application.**

Upon receipt of the application by the PHS, this form will be separated from the application. This form will **not** be duplicated, and it will **not** be a part of the review process. Data will be confidential, and will be maintained in Privacy Act record system 09-25-0036, "Grants: IMPAC (Grant/Contract Information)." The PHS requests the last four digits of the Social Security Number for accurate identification, referral, and review of applications and for management of PHS grant programs. Although the provision of this portion of the Social Security Number is voluntary, providing this information may improve both the accuracy and speed of processing the application. Please be aware that no individual will be denied any right, benefit, or privilege provided by law because of refusal to disclose this section of the Social Security Number. The PHS requests the last four digits of the Social Security Number under Sections 301(a) and 487 of the PHS Acts as amended (42 U.S.C 241a and U.S.C. 288). All analyses conducted on the date of birth, gender, race and/or ethnic origin data will report aggregate statistical findings only and will not identify individuals. If you decline to provide this information, it will in no way affect consideration of your application. Your cooperation will be appreciated.

DATE OF BIRTH (*MM/DD/YY*)	05/23/62	SEX/GENDER
SOCIAL SECURITY NUMBER (last 4 digits only)	XXX-XX- 9480	☒ Female ☐ Male

ETHNICITY

1. Do you consider yourself to be Hispanic or Latino? (See definition below.) Select one.

Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," can be used in addition to "Hispanic or Latino."

☐ **Hispanic or Latino**

☒ **Not Hispanic or Latino**

RACE

2. What race do you consider yourself to be? Select one or more of the following.

☐ ***American Indian or Alaska Native.*** A person having origins in any of the original peoples of North, Central, **or** South America, and who maintains tribal affiliation or community attachment.

☐ ***Asian.*** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian **subcontinent**, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

☐ ***Black or African American.*** A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or African American."

☐ ***Native Hawaiian or Other Pacific Islander.*** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or **other** Pacific Islands.

☒ ***White.*** A **person** having origins in any of the original peoples of Europe, the Middle East, or North Africa.

☐ Check here if you do not wish to provide some or all of the above information.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title:

Total Planned Enrollment:

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino			
Ethnic Category: Total of All Subjects *			
Racial Categories			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American			
White			
Racial Categories: Total of All Subjects *			

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

Principal Investigator/Program Director (Last, First, Middle):

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: _____

Total Enrollment: _____ **Protocol Number:** _____

Grant Number: _____

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
	Sex/Gender			
Ethnic Category	**Females**	**Males**	**Unknown or Not Reported**	**Total**
Hispanic or Latino				**
Not Hispanic or Latino				
Unknown (individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*				*
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of All Subjects*				*

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Unknown or Not Reported**	**Total**
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of Hispanics or				**

* These totals must agree.
** These totals must agree.

CDA TOC Substitute Page Candidate (Last, first, middle):

Use this substitute page for the Table of Contents of Research Career Development Awards. Type the name of the candidate at the top of each printed page and each continuation page.

RESEARCH CAREER DEVELOPMENT AWARD
TABLE OF CONTENTS (Substitute Page)

Page Numbers

Appendix (Five collated sets. No page numbering necessary.) ☐ Check if Appendix is included

Number of publications and manuscripts accepted for publication *(not to exceed 5)* _____

List of Key Items:

Note: Font and margin requirements must conform to limits provided in the Specific Instructions.

*Include these items only when applicable.

CITIZENSHIP

☐ U.S. citizen or noncitizen national ☐ Permanent resident of U.S. (If a permanent resident of the U.S., a notarized statement must be provided by the time of award.)

CAREER DEVELOPMENT AWARD REFERENCE REPORT GUIDELINES
(Series K)

Title of Award:

Type of Award: **Application Submission Deadline:** _____

Name of Candidate (Last, first, middle):

Name of Respondent (Last, first, middle):

The candidate is applying to the National Institutes of Health for a Career Development Award (CDA). The purpose of this award is to develop the research capabilities and career of the applicant. These awards provide up to five years of salary support and guarantee them the ability to devote at least 75–80 percent of their time to research for the duration of the award. Many of these awards also provide funds for research and career development costs. The award is available to persons who have demonstrated considerable potential to become independent researchers, but who need additional supervised research experience in a productive scientific setting.

We would appreciate receiving your evaluation of the above candidate with special reference to:

- potential for conducting research;
- evidence of originality;
- adequacy of scientific background;
- quality of research endeavors or publications to date, if any;
- commitment to health-oriented research; and
- need for further research experience and training.

Any related comments that you may wish to provide would be welcomed. These references will be used by PHS committees of consultants in assessing candidates.

Complete the report in English on 8-1/2 x 11" sheets of paper. Return your reference report to the candidate sealed in the envelope as soon as possible and in sufficient time so that the candidate can meet the application submission deadline. References must be submitted with the application.

We have asked the candidate to provide you with a self-addressed envelope with the following words in the front bottom corner: “DO NOT OPEN—PHS USE ONLY.” Candidates are not to open the references. Under the Privacy Act of 1974, CDA candidates may request personal information contained in their records, including this reference. Thank you for your assistance.

Type the name of the principal investigator/program director at the top of each printed page and each continuation page. (For type specifications, see PHS 398 Instructions.)

INSTITUTIONAL RUTH L. KIRSCHSTEIN NATIONAL RESEARCH SERVICE AWARD TABLE OF CONTENTS (Substitute Page)

* Font and margin requirements must conform to limits provided in PHS 398 Specific Instructions.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY (Kirschstein-NRSA Substitute Page)	**FROM**	**THROUGH**

STIPENDS	**DOLLAR TOTAL**
PREDOCTORAL No. Requested:	
POSTDOCTORAL *(Itemize)* No. Requested:	
OTHER *(Specify)* No. Requested:	
TOTAL STIPENDS →	
TUITION and FEES *(Itemize)*	
TRAINEE TRAVEL *(Describe)*	
TRAINEE RELATED EXPENSES (including Health Insurance)	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Also enter on Face Page, Item 7)*	**$**

BUDGET FOR ENTIRE PROPOSED PERIOD OF SUPPORT
DIRECT COSTS ONLY (Kirschstein-NRSA Substitute Page)

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*		ADDITIONAL YEARS OF SUPPORT REQUESTED							
			2nd		3rd		4th		5th	
PREDOCTORAL STIPENDS	No.		No.		No.		No.		No.	
POSTDOCTORAL STIPENDS										
OTHER STIPENDS										
TOTAL STIPENDS										
TUITION AND FEES										
TRAINEE TRAVEL										
TRAINEE RELATED EXPENSES (including Health Insurance)										
TOTAL DIRECT COSTS										

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* **$**

JUSTIFICATION. For all years, explain the basis for the budget categories requested. Follow the instructions for the Initial Budget Period and include anticipated postdoctoral levels.

DO NOT SUBMIT UNLESS REQUESTED
Competing Continuation Applications
KEY PERSONNEL REPORT

All Key Personnel for the Current Budget Period

Name	Degree(s)	SSN (last 4 digits)	Role on Project (e.g. PI, Res. Assoc.)	Date of Birth (MM/DD/YY)	Months Devoted to Project		
					Cal	Acad	Summer

Mailing address for application

Use this label or a facsimile

All applications and other deliveries to the Center for Scientific Review must come either via courier delivery or via the United States Postal Service (USPS.) Applications delivered by individuals to the Center for Scientific Review will no longer be accepted.

Applications sent via the USPS EXPRESS or REGULAR MAIL should be sent to the following address:

CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
6701 ROCKLEDGE DRIVE
ROOM 1040 – MSC 7710
BETHESDA, MD 20892-7710

NOTE: All applications sent via a courier delivery service (non-USPS) should use this address, but CHANGE THE ZIP CODE TO 20817

The telephone number is 301-435-0715. C.O.D. applications will *not* be accepted.

For application in response to RFA

Use this label or a facsimile

IF THIS APPLICATION IS IN RESPONSE TO AN RFA, be sure to put the RFA number in line 2 of the application face page. In addition, after duplicating copies of the application, cut along the dotted line below and staple the RFA label to the bottom of the face page of the original and place the original on top of your entire package. Failure to use this RFA label could result in delayed processing of your application such that it may not reach the review committee on time for review. ***Do not use*** the label unless the application is in response to a specific RFA. Also, applicants responding to a specific RFA should be sure to follow all special mailing instructions published in the RFA.

RFA No. ____ _____

RFA

Mailing address for application

Use this label or a facsimile

All applications and other deliveries to the Center for Scientific Review must come either via courier delivery or via the USPS. Applications delivered by individuals to the Center for Scientific Review will no longer be accepted.

Applications sent via the USPS EXPRESS or REGULAR MAIL should be sent to the following address:

CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
6701 ROCKLEDGE DRIVE
ROOM 1040 – MSC 7710
BETHESDA, MD 20892-7710

NOTE: All applications sent via a courier delivery service (non-USPS) should use this address, but CHANGE THE ZIP CODE TO 20817

The telephone number is 301-435-0715. C.O.D. applications will *not* be accepted.

For application in response to SBIR/STTR

Use this label or a facsimile

IF THIS APPLICATION IS IN RESPONSE TO AN SBIR/STTR Solicitation, be sure to put the SBIR/STTR Solicitation number in line 2 of the application face page. In addition, after duplicating copies of the application, cut along the dotted line below and staple the appropriate SBIR or STTR label to the bottom of the face page of the original and place the original on top of your entire package. If this SBIR or STTR application is in response to an RFA, be sure to also include the RFA No. in the space provided below.

SBIR

RFA No. _____(if applicable)

STTR

RFA No. _____(if applicable)

Laboratory:

Most research will occur at the Armed Forces Radiobiology Research Institute (AFRRI), 8901 Wisconsin Avenue, Bethesda, MD 20889-5603. Most of the research will occur in the Applied Cellular Radiobiology Department. Basic equipment (work benches, cabinets, sinks, utilities, chemical safety hoods, flammable storage cabinets, and refrigerators/freezers) are available in each laboratory. Additionally, Drs. McClain and Kalinich have over 550 sq. ft. of laboratory spece that contains a dedicated tissue culture facility and microscopy area. The histopathology laboratory in the Veterinary Science Department at AFRRI is a 700 sq. ft. laboratory with the equipment and supplies necessary to process formalin-fixed and frozen tissue sections and a wide variety of common and secial stains available.

Computer:

Additionally, through USUHS site licenses a variety of software applications are available, including: scientific programming languages; spreadsheet, statistical analysis packages; and communications (email), Endnote, and presentation software. The University Information Systems department has a Helpdesk that provides maintenance for desktop machines and training for University students and employees. The central computing facilities include both Digital VAX and Alpha computers. SAS is included on the computers. The university is connected to the Internet via T1 link and has a modem bank. Full access to websites and International email is available. Three computers and printers are located in the research center. A dedicated computer is required for data safety and integrity of the data.

Doctoral Proposal Defense

Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents

Roberta Lavin, MSN, APRN, BC
CAPT, USPHS
Uniformed Services University of the Health Sciences
March 8, 2007

Dissertation Committee

- Chair
  - Christine Kasper, PhD, RN, FAAN, FACSM
    - Professor, USUHS/VA ONS
- Members
  - Roopa Biswas, PhD
    - Assistant Professor, USUHS
  - John Kalinich, PhD
    - Research Biochemist, Armed Forces Radiobiology Research Institute
  - Marguerite Littelton Kearney, PhD, RN, FAAN
    - Associate Professor, John Hopkins Schools of Nursing and Medicine

Purpose of Study

- The purpose of the research is to:
  - Identify early changes in skeletal muscle in Fischer 344 rats that may indicate that heavy metal tungsten alloy (HMTA) is causing muscle damage, and
  - Identify early changes in ultrastructure and morphology of skeletal muscle that may be a precancerous indicator

Innovation

- No studies could be found involving early changes in skeletal muscle after exposure to HMTA
  - Searches included:
    - PubMed
    - CINAHL
    - Biomedical Research Database
    - Federal Research in Progress
    - CRISP

Federal & Military Relevance

- Military and civilian personnel increasingly face shrapnel injuries that may contain HMTA
- As individuals with shrapnel injuries containing unknown metals return home, it is critical that nurses be aware of the potential indications of embedded HMTA
  - This knowledge will provide a basis for policy changes on the removal of shrapnel and a basis on which to extrapolate nursing assessments that will be necessary at the bedside for persons with a history of shrapnel injuries
  - This should lead to further research on clinical care of patients with shrapnel injuries

Background

- Tungsten is a naturally occurring substance
  - Blood level of 1-6 mg/L and urine levels of 0.085 mg/L (ATSDR, 2003)
  - There is some evidence that occupational exposure to dust produced by hard metal industry may result in adverse health effects
    - May be related to cobalt and not tungsten
- Tungsten alloy is increasingly used in military munitions (Kalinich et al., 2005)
- Utilized as a replacement for depleted uranium (DU) in military weaponry, such as bullets (ATSDR, 2003)

Background

- 100% of the rats implanted with HMTA developed pleomorphic rhabdomyosarcoma
- Until the experiments by Miller et al. (2001) and Kalinich et al. (2005) there were few studies identifying the health effects of either oral or dermal exposure to tungsten compounds
- Kalinich et al. investigated the potential health effects of HMTA using a rodent model
- The findings raised serious concerns about the health effects of tungsten/nickel/cobalt alloys in munitions

Background

- No one knows the total number of persons with shrapnel injuries
- As of September 2006 approximately 1900 individuals had been screened by bioassay and approximately 2100 specimen analyzed
  - Based on bioassay analysis 68 fragments from 60 individuals were analyzed further and none contained HMTA
- Shrapnel is generally not removed unless it is either a large fragment or in a location where it is likely to cause further damage

Significance

- Research involving HMTA that has been completed is compelling
- More research is needed that not only confirms the health effects of embedded HMTA, but also identifies any early signs of damage that may indicate precancerous changes
- As more HMTA is used in munitions the health risk to causalities with shrapnel injuries will increase

Overall Objective

- The overall objective of this research study is to provide information about early signs of skeletal muscle changes that may indicate impending adverse consequences of embedded HMTA

Aims and Hypothesis

- Specific Aims
  - Characterize the ultrastructural and morphologic forms of skeletal muscle damage characteristic of the presence of embedded HMTA
    - Hypothesis 1: The characteristics of skeletal muscle damage are significantly different in the F344 rats with embedded HMTA than in F344 rats with embedded Tantalum (Ta)
    - Hypothesis 2: The characteristics of skeletal muscle damage are not significantly different in F344 rats with HMTA than in F344 rats with embedded Nickel (Ni)
    - Muscle damage will be measured by disintegration of muscle fiber, loss of connective tissue, change in vascularity, change in fiber type, change in area, circularity, and change in number of fibers,

Aims and Hypothesis

- Determine if the rate and magnitude of the development of early signs of damage or change in the ultrastructure and morphology of skeletal muscle is a precancerous indicator
  - Hypothesis 3: Skeletal muscle damage in rats with embedded HMTA is present prior to tumor development

Aims and Hypothesis

- Correlate the rate and magnitude of rhabdomyosarcoma development at 3 time points to the morphology and ultrastructure of the experimental muscle
  - Hypothesis 4: Morphology and ultrastructure changes of the muscle with embedded HMTA indicative of skeletal muscle damage will significantly increase as pleomorphic rhabdomyosarcoma develops as measured by tumor size, mitotic rate, and extent of necrosis

Conceptual Models, Frameworks & Theories

- Assumptions
  - Biomedical model presumes that HMTA causes cancer and affects skeletal muscle pathology
  - Data from rodent model will shape knowledge relevant to human pathology
  - The research will help to explain the effects of HMTA on muscle

Multistage Model of Carcinogenicity

- Carcinogenesis is a complex and dynamic biological process that is best viewed as a system
  - This classic theory of cancer is now foundational
  - There are a number of cells (N) that can divide and potentially experience a carcinogenic transformation
  - The *k*th change, which is sudden and irreversible, results in the development of cancer
  - It is assumed that there is a delay between transformation to cancer and actual detection

Physiologic Rodent Model

- The rat is an excellent physiologic model for assessing the effects of embedded metal fragments
  - Used as the model for metal-induced toxicological effects for many years
  - Muscle is histologically similar to humans
  - Extensive toxicological studies provide a good database
  - Available data on HMTA is exclusively in rodents
  - The size of the animal allows the rapid and technically straightforward implantation of multiple pellets without noticeable discomfort to the rat

Physiologic Rodent Model

- An animal model is necessary to completely address the question of histological changes as a pre-carcinogenicity indicator
  - Not possible to base pre-cancer histology assessment on in vitro studies
  - The carcinogenicity process resulting from embedded HMTA is not well understood
- An animal physiologic model is necessary to understand what effect HMTA, when embedded like shrapnel, has on the incidence of cancer and the resulting histological changes

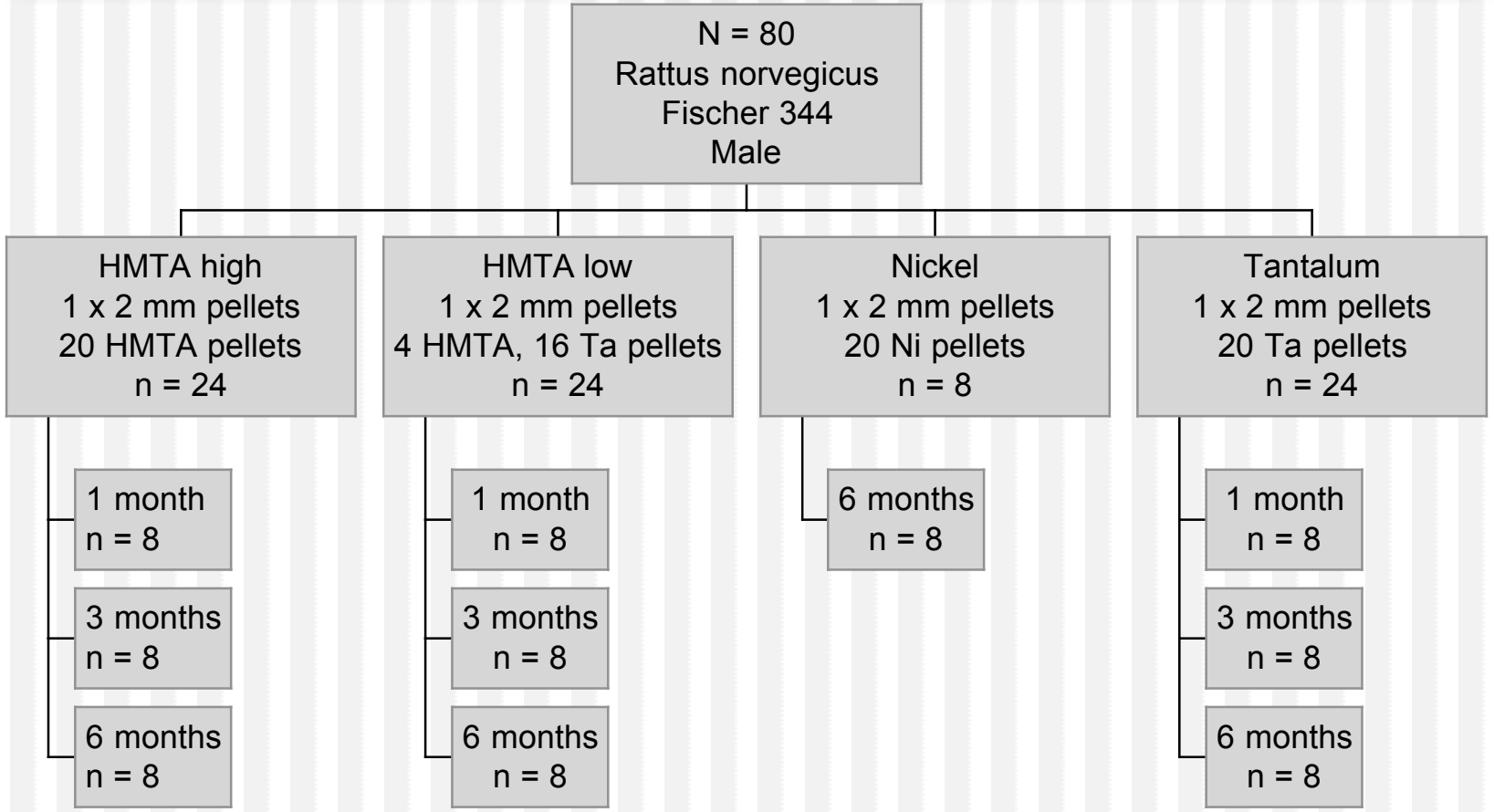
Overview of Study Design

- Previously completed study on F344 male rats
- Implanted between days 63-70
- HMTA pellets consisted of 91.1% tungsten, 6% nickel, and 2.9% cobalt
- All pellets were cylinders 1mm x 2 mm long

Overview of Methods

- Tissue samples will be histologically examined
- Samples from 80 rats will provide histological data for at 3 time points (1, 3, & 6 months)
  - HMTA high and low dose
  - Ta (negative control)
  - Ni high and low dose (positive control) (only at 6 months)

Study Groups



IACUC

- Maintained in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals
- IACUC approval was obtained for the original study (protocol # 0701-ACM-01.0-AID)
- Tissue does not require further IACUC approval

Sample Size and Power

- 10 subjects per group = statistical power of .80
  - Two-sided significance of .05 and effect of 1.325
- 6 subjects per group = statistical power of .80
  - Two-sided significance remains .05 and effect size of 1.796

Limitations

- Laboratory variables and statistical analysis of data is possible with quantitative design allowing for the possibility to accept or reject the hypothesized relation among variable
- However it is not the same as the natural phenomena
- Experimental research in a laboratory may be hard to generalize outside the laboratory and may require translational research in the natural environment

Data Analysis

- Aim 1: Characterization of ultrastructural and morphologic forms of skeletal muscle damage
- Aim 2: Rate and magnitude of early signs of damage
- Aim 3: Rate and magnitude of the development of rhabdomyosarcoma

Data Analysis

- Micrographs of muscle cross-sections will be analyzed using ImageJ
  - Image J is a public access image process program based on NIH Image
  - Designed to allow detailed quantitative analysis
  - Widely used in a range of measurement applications
  - Has been show to be a valid and reliable alternative to manual measures (Tran, 2000)
  - All data can be statistically evaluated as scale data in SPSS

Statistical Analysis

- Muscle fiber size varies
  - Age, sex, and weight
  - All male rats are older than 81 days at the time of sampling
- Standard fiber type assessment procedures of Armstrong & Phelps (1984) will be followed
- Descriptive statistics will be used to determine SD of all variables acquired from cross-sections
    - Data will be presented as mean ± SEM
- Differences between the groups will be analyzed using a two-way ANOVA
  - Significant F ratio: differences will be located using LSD post hoc analysis for planned comparisons

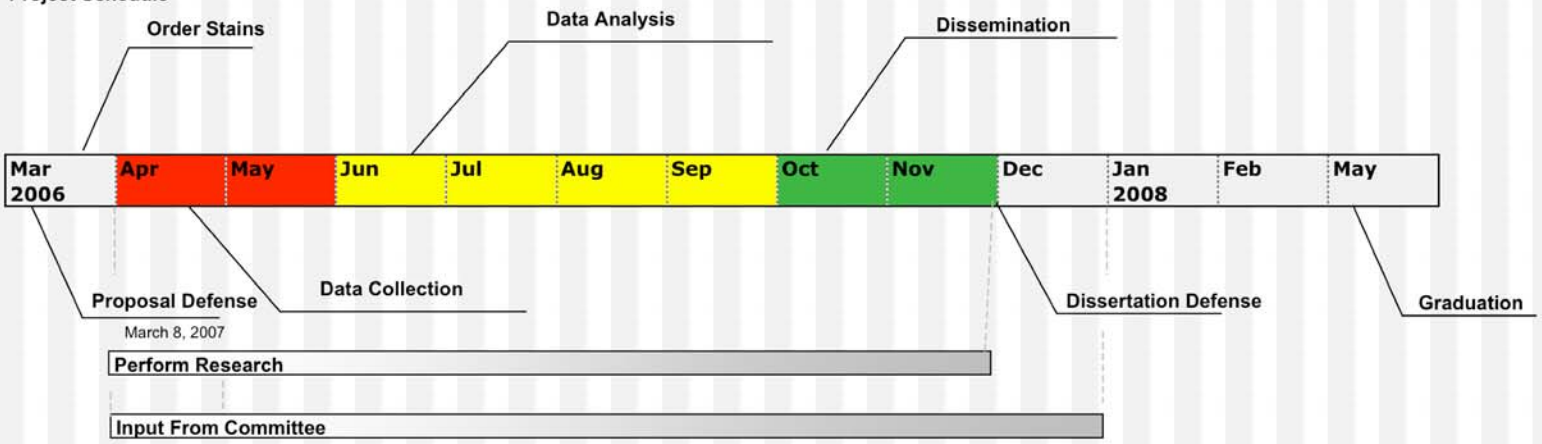
Dissertation Timeline

vertex^42 **Dissertation Timeline**

CAPT Roberta Lavin

Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents

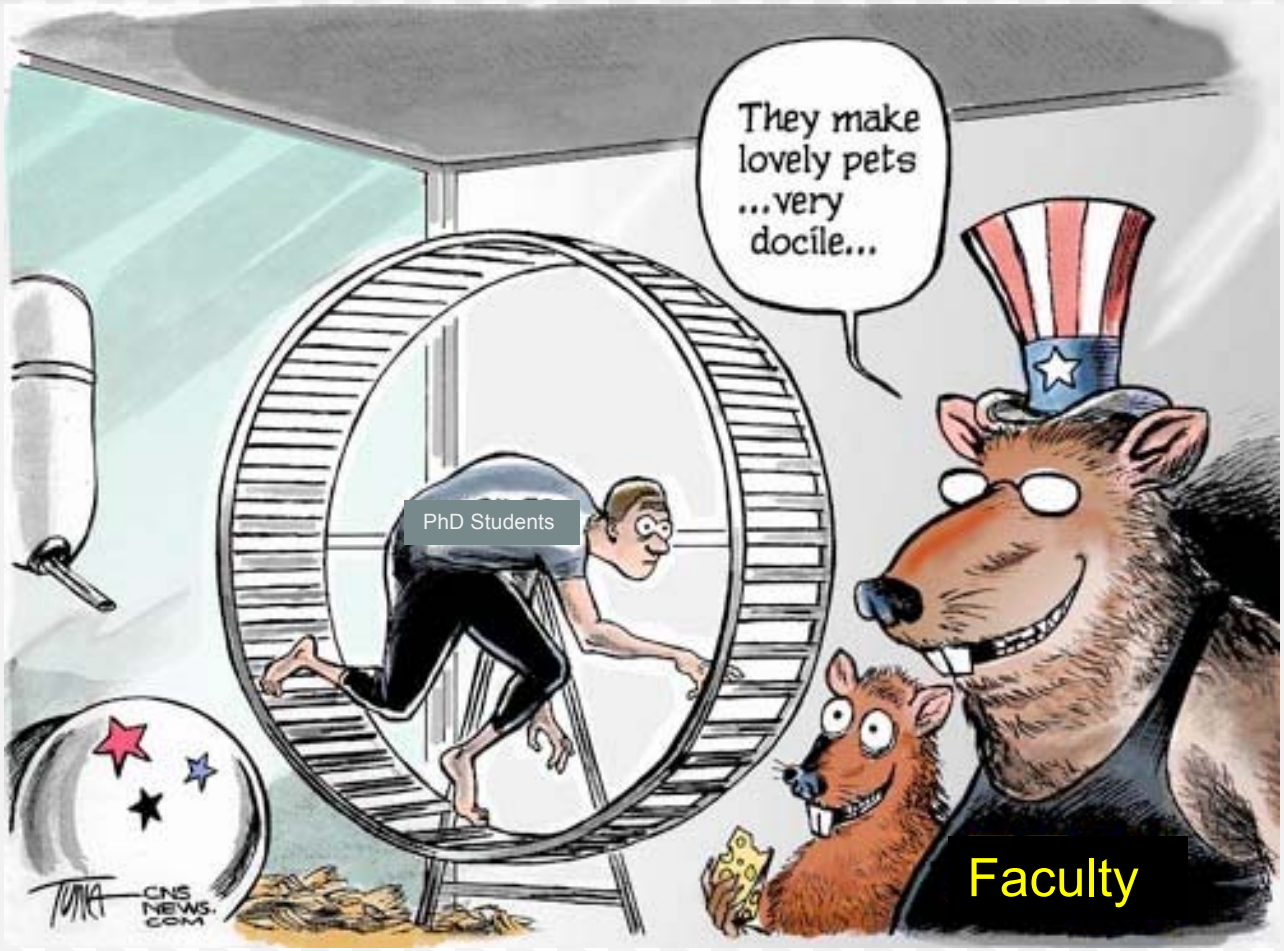
Project Schedule



Future Research

- Longitudinal study
- Clinical care of patients with shrapnel injuries
- Rodent studies of other forms of shrapnel injuries and the histologic changes
- Policy analysis

Questions


They make lovely pets ...very docile...
PhD Students
Faculty
TOMKA
CNS NEWS.COM

Measures of Muscle Damage

- Fiber damage can be evaluated using anti-desmin, anti-vimentin and anti dystophin (Martins et al., 2006, Putman et al., 2001). Through utilization of the procedures of Martins muscle fiber damage can be identified by vimentin positive, dystrophin negative, and an altered pattern of desmin staining, for example areas without staining or with foci of positivity.

Uniformed Services University of the Health Sciences
Graduate School of Nursing
Request for Appointment of Dissertation Chairperson (Form C)

Name of Student: CAPT Roberta Lavin

Semester: Spring 2007 Area of Concentration: Physiological – Bench Science

Name of Selected Dissertation Chairperson: Dr. Christine E. Kasper

Phone Number 202 422 2679

The above named student has selected the named faculty member to serve as Dissertation Chairperson.

The undersigned faculty member agrees to serve as the Dissertation Chairperson, understanding all responsibilities that are part of this critical role:

Christine Kasper
Printed Name

Signature

Roberta Lavin
Printed Name of Student

Signature

Approval/Disapproval

Signature: Karen Elberson Date: 8 March 2007
Karen Elberson, RN, PhD
Director, Doctoral Program

Approval/Disapproval

Signature: Date: 8 Mar 07
William T. Bester, RN, MSN, CNAA, BC
Brigadier General (Ret)
Acting Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing
Request for Appointment of Dissertation Advisory Committee (Form D)

Name of Student: CAPT Roberta Lavin

Semester: Spring 2007 Area of Concentration: Physiological – Bench Science

Dissertation Chairperson: Dr. Christine Kasper

Selected Faculty to Serve as Dissertation Advisory Committee:

1. Dr. Roopa Biswas Phone # 295-1009
2. Dr. John Kalinich Phone # 295-9242
3. Dr. Marguerite Littleton-Kearney Phone # 443-287-0179

The above named student has selected the named faculty members to serve as the Dissertation Advisory Committee.

The undersigned faculty members agree to serve as the Dissertation Advisory Committee, understanding all responsibilities that are part of this critical role:

Roopa Biswas — Roopa Biswas
Printed Name of Faculty Member — Signature

John Kalinich — [signature]
Printed Name of Faculty Member — Signature

Marguerite Littleton-Kearney — Marguerite Littleton-Kearney
Printed Name of Faculty Member — Signature

Roberta Lavin — [signature]
Printed Name of Student — Signature

(Approval)/Disapproval

Signature: Karen Elberson Date: 8 March 2007
Karen Elberson, RN, PhD
Director, Doctoral Program

(Approval)/Disapproval

Signature: [signature] Date: 8 MAR 07
William T. Bester, RN, MSN, CNAA, BC
Brigadier General (Ret)
Acting Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing
Report of Proposal Defense Examination
for the Doctor of Philosophy Degree (Form E)

The proposal defense of CAPT Roberta Lavin,

entitled: *Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents*
was held on 8 March 2007 from 1000 to 1100.

The decision of the Examining Committee is:

PASS
A. Both the proposal and the oral explanation are satisfactory: ✓ CEK

B. Minor changes are recommended by the Dissertation Advisory Committee and are to be made to the satisfaction of the Dissertation Chairperson: _____

DEFER
A. Major changes in the proposal are required. Changes must be made to the satisfaction of the Dissertation Chairperson: _____

B. Major changes are required. Changes must be made to the satisfaction of the Dissertation Advisory Committee: _____

C. Remediation required prior to making major changes. Completion of remediation must meet the satisfaction of the Dissertation Advisory Committee: _____

FAIL
Neither the oral performance nor the proposal is adequate: _____

Signatures of the Committee

Chairperson: Christine E Kasper

Member: Roopa Biswas.

Member: Marguerite Littleton-Kearney

Member: [illegible]

(Approval)/Disapproval

Signature: Karen Elberson
Karen Elberson, RN, PhD
Director, Doctoral Program

Date: 8 March 2007

(Approval)/Disapproval

Signature: William T. Bester
William T. Bester, RN, MSN, CNAA, BC
Brigadier General (Ret)
Acting Dean, Graduate School of Nursing, USUHS

Date: 8 MAR 07

Running Head: Modern Hazards

Effect of Heavy Metals on Skeletal Muscle: Shrapnel Injuries as a Modern Hazard

CAPT Roberta Proffitt Lavin

Uniformed Services University

Graduate School of Nursing

4301 Jones Bridge Road

Bethesda, Maryland 20814-4799

Co- Authors:

Christine E. Kasper, PhD, RN, FAAN

John F. Kalinich, PhD

Abstract

The debate over whether to remove embedded shrapnel is reemerging as a result of continued research into the health effects of embedded heavy metal fragments. Reports of rhabdomyosarsoma in rats embedded with heavy metal tungsten alloy appeared in the literature in 2005. An etiological relationship that is strongly suspected by some is still in question. Heavy metal tungsten alloy is one of the newest compounds to enter the munitions of militaries. It was chosen as a safe replacement for depleted uranium, which is believed to have long-term adverse health effects. The study of tumorigenicity and carcinogenicity of all forms of heavy metals utilized in munitions provides a general understanding of long-term adverse health effects of shrapnel injuries.

The views expressed in this article are those of the author and do not necessarily reflect those of the U.S. Department of Health and Human Services or the Uniformed Services University of the Health Sciences. There is no conflict of interest in this publication. (Comment of any funding)

It has been estimated that over 28451 Americans were wounded in Iraq since March 2003 ("Operation Iraq Freedom (OIF) Casualty Status," 2007; White, 2007). Fragmentation wounds accounted for approximately forty-nine percent of wounds in the Persian Gulf War and forty-six percent of wounds in the first phase of Operation Iraqi Freedom (Burkle, Newland, Meister, & Blood, 1994; Zouris, Walker, Dye, & Galarmeau, 2006). While a paucity of literature exist on the percentage of retained fragments from fragmentation wounds Hamouda (2007) found that one-third of patients had retained missile fragments, which if predictive of missile wounds in general would indicate that approximately fifteen percent of those wounded will carry shrapnel in their bodies for the rest of their lives. For most, the shrapnel will have caused its damage at the time of the injury and the soldier[1] will suffer no further harm. However, in addition to causing skeletal muscle pathology, munitions composed of heavy metals, such as depleted αuranium and tungsten alloys, have chemical properties that are also carcinogenic.

The purpose of this article is to review the existing literature on the effects of embedded heavy metals and ionizing radiation on skeletal muscle including the recent findings that tungsten alloy may be linked to malignant rhabdomyosarcoma in rodents. Relevant reviews of the health effects of embedded foreign bodies, lead, depleted uranium (DU), and exposure to radiation sources will help to clarify that it is important to identify the composition of embedded shrapnel in order to predict the potential long-term health consequences. This limited though growing body of literature may promote a review of the existing practice of leaving shrapnel embedded and the importance of

---

[1] Soldier will be used to refer to all military personnel from all branches of service.

identifying the composition of the fragments since the health effects from different heavy metals varies.

Foreign Body Tumorigenesis

There is ample evidence from the literature that shrapnel injuries, while infrequent, can continue to cause health complications decades after the initial injury (Dillman, Crumb, & Lidsky, 1979; Knox & Wilkinson, 1981; Ligtenstein, Krijnen, Jansen, & Eulderink, 1994; Lindeman, McKay, Taubman, & Bilous, 1990; Schenck & Kronman, 1977; Symonds, Mackay, & Morley, 1985). Animal models have been used to show how different types of embedded fragments can result in tumors (Thomassen, Buoen, Brand, & Brand, 1978; Thomassen, Buoen, & Brand, 1975). However, the development of serious complications, such as tumors in humans, is rarely reported in the literature.

Any foreign body has the potential for tumorigenesis. The physical presence and nature of a foreign body -- not the chemical reactivity -- are primarily responsible for tumor development in subcutaneous and intraperitoneal implants (Brand, Buoen, Johnson, & Brand, 1975). Brand et al. (1975) implanted mice with various glass and plastic films to study the combined effects of foreign bodies and chemicals. They disproved prior suggestions that tumorigenesis by vinyl chloride vinyl acetate copolymer implants involved both foreign body and chemical carcinogenesis and suggested that in the absence of demonstrable chemical carcinogenic activity foreign body tumorigenesis must be assumed.

The vast majority of foreign body experiments involve implantation in epithelial-lined organs, inhalation, and subcutaneous injections (Brand, Johnson, & Buoen, 1976). (For a detailed account of foreign body tumorigenesis, see the review by Brand, Johnson, and Buoen of 1976. For a review of carcinogenicity of metal alloys, see

Sunderman of 1989). The vast majority of knowledge about the carcinogenicity of heavy metals comes from animal studies (Gaechter et al., 1977). In such animal studies it has been shown that compounds containing iron (Fe), lead (Pb), nickel (Ni), and other metals appear to be carcinogenic (Rigdon, 1974; Sunderman, 1989).

Heavy Metals

Lead

Lead was one of the first heavy metals used to make munitions and continues to be a source of exposure, mostly to persons who make their own bullets (American Cancer Society, 2003). Like other heavy metal sources, lead exposure primarily occurs through occupational and environmental means (Agency for Toxic Substances and Disease Registry, 2005). While there is evidence that links lead to increased risk of brain cancer (Cocco, Dosemeci, & Heineman, 1998; Lundstrom et al., 1997), there is none linking lead to skeletal muscle tumors. In fact, there are few reported cases of adverse affects from embedded lead bullets in the literature; however, one of the findings reported is inflammation (McQuirter et al., 2004).

Case studies of persons with gunshot wounds with retained lead fragments have consistently shown significantly elevated blood lead levels for months and adverse health effects for decades (Dillman et al., 1979; Gerhardsson, Dahlin, Knebel, & Schutz, 2002; Magos, 1994; Stromberg, 1990). It is believed that a blood lead level > 30μg/dL, which is greater than 10 times the population mean, affects the nervous system and may impair recovery (Gerhardsson et al., 2002; McQuirter et al., 2004). The large number of gunshot wounds in the U.S. and the paucity of articles in the literature about tumors associated with gunshot wounds is an indication that tumorigenesis from retained lead fragments is rare.

Depleted Uranium

In order to understand DU's potential toxicity, we must consider several of its characteristics, including its chemical form, isotopic mixture, and particle size. Uranium, as found in nature, consists primarily of three isotopes in the following percentages (by weight): ^{238}U (99.282%), ^{235}U (0.711%), and ^{234}U (0.005%). As produced for power generation and nuclear weapons, uranium, which contains greater than 0.711% ^{235}U, is considered "enriched" uranium. DU, obtained as a byproduct of the isotope enrichment process that produces high-specific activity uranium, contains the following isotopes (by weight): ^{238}U, 99.8%; ^{235}U, 0.2%; and ^{234}U, 0.001%. Thus, DU has a specific activity significantly less than natural uranium (0.4 µCi/g for DU versus 0.7 µCi/g for natural uranium). However, there are no differences in their chemical characteristics (Daxon & Musk, 1993). DU is used by the military to fabricate armor and kinetic energy penetrators. Its effectiveness as a penetrator is a consequence of its very high density (approximately 1.7-times that of lead: 19 g/cm^3 versus 11.35 g/cm^3) and its pyrophoric character. This results in its ignition under conditions of extreme temperature and pressure (such as that which occur upon impact with armored targets).

While DU's radioactivity is reduced, it is not eliminated. Like natural uranium, DU emits alpha, beta, and weak gamma radiation (McClain et al., 2001). DU presents a minimal external radiation hazard, because the alpha particles emitted cannot penetrate the dead layer of skin (approximately 20 microns). The beta radiation is hazardous only if extended contact occurs and the amount of the more penetrating gamma radiation is low (< 1% of total radiation). DU internalized as a result of ingestion, inhalation, or embedded shrapnel fragments, represents an uncertain hazard. The health risks from

such exposures may occur as a result of chemical toxicity, chronic radiation exposure, or both (North Atlantic Treaty Organization, 2005).

Reports by the World Health Organization (2003) and the Institute of Medicine (2000) reported that neither civilian nor military DU exposure was likely to exceed normal background levels. For those that do have significant exposure the most likely health effects would be to the kidneys and the lungs. In occupation epidemiology studies of uranium miners and processors the only long-term effect was decreased kidney function. Lung cancer in uranium workers was attributed to smoking and radon gas and not uranium. The epidemiology studies that have been completed cite problems with confounding variables such as smoking and multiple chemical exposures as introducing possible bias (Ritz, Morgenstern, Crawford, & Young, 2000). (For a full review of the literature on occupational exposure to uranium from minning, processing and work at nuclear facilities see the Institute of Medicine Report, Gulf War and Health: Volume 1. Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines, 2000).

As a result of friendly fire incidents, several soldiers from the first Gulf War are carrying fragments of DU in their bodies. These veterans have persistently elevated levels of uranium in their urine although no other adverse health effects have been reported (McDiarmid et al., 2004; McDiarmid et al., 2000; McDiarmid et al., 2001). In experimental models, embedded DU has been shown to relocate and deposit in the kidneys and bone, as well as affect oncogene expression and decrease fertility (NATO, 2005). DU also has both transforming (Miller, Blakely et al., 1998) and mutagenic properties (Miller, Fuciarelli et al., 1998). It has been shown that tumorigenic potential can be conferred on a human osteoblastic sarcoma cell line exposed to soluble and insoluble DU compounds (Miller, Blakely et al., 1998). To determine if the hazardous

effects of DU were due to radiation or chemical effects, Miller et al. (2002) incubated human osteoblast cells with DU and nonradioactive heavy metals and processed them for dicentric analysis -- an indicator of radiation-induced DNA damage. It was concluded that hazards from DU are likely due, in part, to radiation even though there is low specific activity (O.44 $\mu Ci.g^{-1}$).

The finding that DU can induce both chemical and radiation toxicity raises concern as to the wisdom of leaving such fragments in place. Most biomolecules including deoxyribonucleic acid (DNA), lipids, and proteins are of ionizing radiation (Terato, Tanaka, Nakaarai, Furusawa, & Ide, 2004). Exposure to ionizing radiation can be lethal, resulting in apoptosis of the cell. Non-lethal changes can occur at lower doses and include: a) delayed mitosis causing changes in cell kinetic patterns, b) disruptions in cell growth, c) increased or decreased permeability resulting from a change in the lipid bilayers due to a loss of metabolic equilibrium, and d) decreased cell motility (NATO, 2005). It follows that the cells most sensitive to radiation are those that are actively proliferating. For example, hemocytoblasts and lymphocytes are very sensitive to radiation. Likewise, the cells that are most radioresistant are those that do not normally divide, such as striated muscle cells. Skeletal muscle is listed among the cells that are the least radiosensitive.

Despite the fact that skeletal muscle is less radiosensitive than most cells, there is evidence that it is impacted by radiation exposure. It has been shown that skeletal muscle cells exposed to low doses of ionizing radiation lose the ability to generate passive and active tension in response to stretch and calcium. This loss of stretch is believed to be caused by the loss of the cytoskeletal proteins titin and nebulin (Horowits, Kempner, Bisher, & Podolsky, 1986).

A 25 Gy dose of gamma radiation has been shown to prevent myofiber hypertrophy in rodent skeletal muscle. Irradiation did not alter fast-to-slow fiber type adaptation and was independent of satellite cells (Rosenblatt & Parry, 1992). Studies using three 25 Gy doses of gamma irradiation (Martins et al., 2006) and one 60 Gy dose of ionizing radiation (Lewis, 1954) did not produce skeletal muscle damage. However, Martins et al. (2006) found that three 25 Gy doses of gamma radiation completely ablated the satellite cell population resulting in mitotic failure and apoptosis. The 25 Gy dose over 3 weeks was considered to be critical in preventing myopathy, while killing satellite cells. While embedded DU will not result in a dose of anywhere near 25 Gy, it will deliver a chronic low dose to muscle cells adjacent to the fragment. This effect has not been thoroughly investigated.

Tungsten Alloy

Tungsten has been used for many years in a variety of applications. Chemically similar to molybdenum, it has been used experimentally as an epileptogenic agent (Ito, Hori, Yoshida, & Shimizu, 1980; Kusske, Wyler, & Ward, 1974; Schwindt, Spain, & Crill, 1984) and to induce molybdenum deficiency in laboratory animals (Fleshman, Krokz, & Silva, 1966). Combining the hard, brittle tungsten metal (W) with various other metals produces heavy-metal tungsten alloys (HMTA) with specific characteristics of interest to the military. HMTAs for military use are usually composed of tungsten (90-97%), nickel (1-5%), and either cobalt (Co) (1-4%) or iron (1-4%) (Lowden & Kelly, 1997). Recently HMTAs have replaced lead in some small-caliber ammunition (so-called “green bullets” (Bogard, Yuracko, Murray, Lowden, & Vaughn, 1988)) and DU in kinetic-energy penetrators (Kerley et al., 1996; Stevens & Lang, 1994). Despite its many uses, very little is known about the health effects of internalized tungsten. The majority of data

come as a result of industrial exposures, particularly hard-metal disease, and from biokinetic experiments using radioactive tungsten. Tungsten is rapidly absorbed (up to 25 times faster than DU) by the body, regardless of the route of exposure (Leggett, 1997). The biokinetics of tungsten are very similar to uranium with respect to its main deposition sites, pathways of movement through the body, and excretion rates (Leggett, 1997). Once in the bloodstream, tungsten is distributed throughout the body before being excreted via the kidney in the urine. Prevailing theory is that elemental tungsten or insoluble tungsten compounds have only limited toxicity (Agency for Toxic Substances and Disease Registry, 2003; Kazantzis, 1986; Kerwien, 1996; Lauring & Wergeland, 1970; Stokinger, 1981). Investigations on hard-metal disease have shown that either tungsten carbide or cobalt alone has limited toxicity on lung tissue (Lasfargues, Lison, Maldague, & Lauwerys, 1992). However, when combined, the tungsten carbide/cobalt mixture acts synergistically to increase the observed toxicity. It is not known whether this is due to the combined toxicity of the tungsten carbide/cobalt mixture or to an increase in the bioavailability of the known toxicant, cobalt (Lison & Lauwerys, 1997).

Information on the health effects of embedded fragments of HMTA is limited. Studies on tungsten-containing waterfowl shot showed no adverse health effects when embedded into the breast muscle of mallards (Kraabel, Miller, Getzy, & Ringelman, 1996). However, the tungsten alloys used as replacements for lead in waterfowl shot are composed of 40-70% tungsten, while HMTAs for military use contain greater than 90% tungsten. Miller et al. (2001) studied the transforming potential of two types of HMTA (W/Ni/Co and W/Ni/Fe) on the human osteoblast-like cell line (HOS) and found that both HMTAs have the ability to transform human cells to the neoplastic phenotype.

A recent study investigating the health effects of a militarily-relevant HMTA (92%W/5% Ni/3% Co) in a rodent model showed that, when embedded into the hind legs of rats, this particular HMTA induced tumors in 100% of the animals (Kalinich et al., 2005). Animals implanted with tantalum, an inert metal, did not develop tumors. The tumors were classified as rhabdomyosarcomas (RMS) and were highly aggressive, metastasizing to the lungs. The link between HMTA and RMS was not predicted. Rhabdomyosarcoma is a highly malignant soft-tissue tumor common in childhood and adolescence, yet rare in adults. There are only about 8000 new soft tissue sarcoma cases identified each year in the United States (Koea, Leung, Lewis, & Brennan, 2003). Horn and Enterline first recognized the histologic subdivisions of RMS in 1958 (Horn & Enterline, 1958); which have been refined over time to include: embryonal, botryoid, alveolar, and pleomorphic subclassifications (Maurer, Ruymann, & Pochedly, 1991; Parham, 2001). The four subtypes of RMS are linked through their mesenchymal origin and the formation of neoplastic skeletal muscle (Maurer et al., 1991; Parham, 2001). As more and more different types of HMTA are used in munitions, the health risk for casualties from shrapnel will increase.

Reasons for Concern

The findings of Kalinich et al. (2005) raise serious concerns about the health effects of tungsten/nickel/cobalt alloys in munitions. Shrapnel is generally not removed unless it is either a large fragment or in a location where it is likely to cause further damage (Peyser, Khoury, & Liebergall, 2006). In response to the findings reported by Kalinich et al. (2005), some metal fragments were removed from U.S. soldiers in Iraq to determine if they contained HMTA. As of September 2006, approximately 1900 individuals had been screened by bioassay and approximately 2100 specimens had

been analyzed. Analysis of 68 fragments from 60 individuals showed that none contained HMTA (DoD, 2005; USACHPPM, 2006). Three fragments from two individuals did contain DU.

Of the studies on HMTA undertaken thus far, none address the effects of the removal of shrapnel on muscle function or the histopathology of surrounding tissue. Given the apparent lethal toxicity of HMTA, it is imperative that a "time window" be established in which embedded HMTA should be removed to prevent the carcinogenic response. Shrapnel injuries not involving HMTA can also result in tumor formation and other long-term health consequences (Knox & Wilkinson, 1981; Ligtenstein et al., 1994; Lindeman et al., 1990; Schenck & Kronman, 1977; Symonds et al., 1985). Thus, future research should focus on two areas: first, the assessment of the health effects of embedded fragments, especially the unique materials found on today's battlefields, and second, the *in situ* identification of shrapnel using blood or urine biomarkers. Given that information, one can make a more informed decision whether to remove shrapnel or to leave it in place.

The potential expanded use of HMTA in munitions and the introduction of new materials, such as explosively-shaped charges on the battlefield, increase the risk of shrapnel wounds in combatants and noncombatants alike (Partlow, 2007). It has been estimated that 60 – 70 % of all wounds result in musculoskeletal injuries (Peyser et al., 2006). The knowledge of the long-term consequences of shrapnel injuries is no longer an issue for military health care alone, but also has a public health impact. Timely and proper care is critical for those with shrapnel injuries. Nurses are the first line of health care and, as such, must carefully monitor patients with shrapnel injuries and be aware of the potential long-term health consequences these situations present.

References

Agency for Toxic Substances and Disease Registry. (2003). *Draft toxicological profile for tungsten*. Retrieved November 29, 2005. from www.astdr.cdc.gov/toxprofiles/tp186.pdf

Agency for Toxic Substances and Disease Registry. (2005). Public Health Statement: Lead. Retrieved September 29, 2007, from http://www.atsdr.cdc.gov/toxprofiles/tp13-c1-b.pdf

American Cancer Society. (2003). Lead. Retrieved September 29, 2007, from http://www.cancer.org/docroot/PED/content/PED_1_3X_Lead.asp?sitearea=PED

Bogard, J. S., Yuracko, K. L., Murray, M. E., Lowden, R. A., & Vaughn, N. L. (1988). *Application of life cycle analysis: the case of green bullets* (No. ORNL/CP-98265): Oak Ridge National Lab.

Brand, K. G., Buoen, L. C., & Brand, I. (1975). Brief Communication: Foreign-body tumorigenesis by Vinyl Chloride Vinal Acetate Copolymer: No evidence for chemical carcinogenesis. *Journal of the National Cancer Institute, 54*(5), 1259-1262.

Brand, K. G., Buoen, L. C., Johnson, K. H., & Brand, I. (1975). Etiological factors, stages, and the role of the foreign body in foreign body tumorigenesis: A review. *Cancer Research, 35*, 279-286.

Brand, K. G., Johnson, H. J., & Buoen, L. C. (1976). Foreign body tumorigenesis. *CRC Critical Review of Toxicology*, 208-393.

Burkle, F., Newland, C., Meister, S., & Blood, C. (1994). Emergency medicine in the Persian Gulf War Part 3: Battlefield casualties. *Annals of Emergency Medicine, 23*(4), 755-760.

Cocco, P., Dosemeci, M., & Heineman, E. F. (1998). Brain cancer and occupational exposure to lead. *J Occup Environ Med, 40*(11), 937-942.

Daxon, E. G., & Musk, J. H. (1993). *Assessment of the risks from embedded fragments of depleted uranium.* (No. Technical Report 93-1). Bethesda: AFRRI.

Dillman, R. O., Crumb, C. K., & Lidsky, M. J. (1979). Lead poisoning from a gunshot wound. Report of a case and review of the literature. *Am J Med, 66*(3), 509-514.

DoD. (2005). *Fact sheet: Tungsten/nickel/cobalt alloy study*. Retrieved Decembe 1, 2005. from www.pdhealth.mil/library/fact_sheets.asp

Fleshman, D., Krokz, S., & Silva, A. (1966). The metabolism of elements of high atomic number. University of California Radiation Laboratory.

Gaechter, A., Alroy, J., Andersson, G. B., Galante, J., Rostoker, W., & Schajowicz, F. (1977). Metal carcinogenesis: a study of the carcinogenic activity of solid metal alloys in rats. *J Bone Joint Surg Am, 59*(5), 622-624.

Gerhardsson, L., Dahlin, L., Knebel, R., & Schutz, A. (2002). Blood lead concentration after a shotgun accident. *Environ Health Perspect, 110*(1), 115-117.

Hamouda, H., Witse, E., Moghani, N., Shahwan, A., & Nygaard, O. (2007). Soft tissue infection after missile injuries to the extremities - A non-randomized, prospective study in Gaza City. *Prehospital and Disaster Medicine, 22*(2), 106-108.

Horn, R. C., & Enterline, H. T. (1958). Rhabdomyosarcoma; a clinicopathologic study and classification of 39 cases. *Cancer, 11*(181).

Horowits, R., Kempner, E. S., Bisher, M. E., & Podolsky, R. J. (1986). A physiological role for titin and nebulin in skeletal muscle. *Nature, 323*(6084), 160-164.

Institute of Medicine. (2000). *Gulf War and Health: Volume 1. Depleted Uranium, Pyridostigmine Bromide, Sarine, and Vaccines*. Washington, DC: The National Academies Press.

Ito, T., Hori, M., Yoshida, K., & Shimizu, M. (1980). Facilitation of tungstic acid gel-induced cortical epilepsy in rats by depression of the reticular formation. *Arch Int Pharmacodyn Ther, 245*(2), 271-282.

Kalinich, J. F., Emond, C. A., Dalton, T. K., Mog, S. R., Coleman, G. D., Kordell, J. E., et al. (2005). Embedded weapons-grade tungsten alloy shrapnel rapidly induces metastatic high-grade rhabdomyosarcomas in F344 rats. *Environ Health Perspect, 113*(6), 729-734.

Kazantzis, G. (1986). Tungsten. In L. Friberg, F. F. Nordberg & V. B. Vouk (Eds.), *Handbook on the Toxicology of Metals* (pp. 610-622). New York: Elsiver.

Kerley, C. R., Easterly, C. E., Eckerman, K. F., Jones, T. D., Leggett, R. W., Munroe, N. B., et al. (1996). *Environmental acceptability of high-performance alternatives for depleted uranium penetrators* (No. ORNL/TM-13286): Oak Ridge National Labs.

Kerwien, S. C. (1996). *Toxicity of tungsten, mlybdenum, and tantalum and the environmental and occupational laws associated with their manufacutre, use, and disposal* (Special Publication No. ARAED SP-96002): U.S. Army Armament Research, Development and Engineering Center, Picatinny Arsenal.

Knox, J., & Wilkinson, A. (1981). Shrapnel presenting with symptoms 62 years after wounding. *Br Med J (Clin Res Ed), 283*(6285), 193.

Koea, J. B., Leung, D., Lewis, J. J., & Brennan, M. F. (2003). Histopathologic type: an independent prognostic factor in primary soft tissue sarcoma of the extremity? *Ann Surg Oncol, 10*(4), 432-440.

Kraabel, B. J., Miller, M. W., Getzy, D. M., & Ringelman, J. K. (1996). Effects of embedded tungsten-bismuth-tin shot and steel shot on mallards. *J Wildlife Dis, 32*, 1-8.

Kusske, J. A., Wyler, A. R., & Ward, A. A., Jr. (1974). Tungstic acid gel as a focal epileptogenic agent. *Exp Neurol, 42*(3), 587-592.

Lasfargues, G., Lison, D., Maldague, P., & Lauwerys, R. (1992). Comparative study of the acute lung toxicity of pure cobalt powder and cobalt-tungsten carbide mixture in rat. *Toxicol Appl Pharmacol, 112*, 41-50.

Lauring, L., & Wergeland, F. L. (1970). Ocular toxicity of newer industrial metals. *Military Medicine, 135*, 1171-1174.

Leggett, R. W. (1997). A model of the distribution and retention of tungsten in the human body. *Sci Total Environ, 206*, 147-165.

Lewis, R. B. (1954). Changes in striated muscle following single intense doses of x-rays. *Lab Invest, 3*(1), 48-55.

Ligtenstein, D. A., Krijnen, J. L., Jansen, B. R., & Eulderink, F. (1994). Forgotten injury: a late benign complication of an unremoved shrapnel fragment--case report. *J Trauma, 36*(4), 580-582.

Lindeman, G., McKay, M. J., Taubman, K. L., & Bilous, A. M. (1990). Malignant fibrous histiocytoma developing in bone 44 years after shrapnel trauma. *Cancer, 66*(10), 2229-2232.

Lison, D., & Lauwerys, R. (1997). Study of the mechanism responsible for the elective toxicity of tungsten-carbide-cobalt powder toward macrophages. *Toxicol Lett, 60*, 203-210.

Lowden, R., & Kelly, R. (1997). *Application of powder metallurgy techniques for the development of non-toxic ammunition* (No. Y/AMT-477): Oak Ridge National Lab.

Lundstrom, N. G., Nordberg, G., Englyst, V., Gerhardsson, L., Hagmar, L., Jin, T., et al. (1997). Cumulative lead exposure in relation to mortality and lung cancer morbidity in a cohort of primary smelter workers. *Scand J Work Environ Health, 23*(1), 24-30.

Magos, L. (1994). Lead poisoning from retained lead projectiles. A critical review of case reports. *Hum Exp Toxicol, 13*(11), 735-742.

Martins, K. J., Gordon, T., Pette, D., Dixon, W. T., Foxcroft, G. R., Maclean, I. M., et al. (2006). Effect of satellite cell ablation on low-frequency-stimulated fast-to-slow fibre-type transitions in rat skeletal muscle. *J Physiol, 572*(Pt 1), 281-294.

Maurer, H. M., Ruymann, F. B., & Pochedly, C. (1991). *Rhabdomyosarcoma and related tumors in children and adolescents*. Boston: CRC Press.

McClain, D. E., Benson, K. A., Dalton, T. K., Ejnik, J., Emond, C. A., Hodge, S. J., et al. (2001). Biological effects of embedded depleted uranium (DU): summary of armed forces radiobiology research institute research. *Sci Total Environ, 274*(1-3), 115-118.

McDiarmid, M. A., Engelhardt, S., Oliver, M., Gucer, P., Wilson, P. D., Kane, R., et al. (2004). Health effects of depleted uranium on exposed Gulf War veterans: a 10-year follow-up. *J Toxicol Environ Health A, 67*(4), 277-296.

McDiarmid, M. A., Keogh, J. P., Hooper, F. J., McPhaul, K., Squibb, K., Kane, R., et al. (2000). Health effects of depleted uranium on exposed Gulf War veterans. *Environ Res, 82*(2), 168-180.

McDiarmid, M. A., Squibb, K., Engelhardt, S., Oliver, M., Gucer, P., Wilson, P. D., et al. (2001). Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. *J Occup Environ Med, 43*(12), 991-1000.

McQuirter, J. L., Rothenberg, S. J., Dinkins, G. A., Kondrashov, V., Manalo, M., & Todd, A. C. (2004). Change in blood lead concentration up to 1 year after a gunshot wound with a retained bullet. *Am J Epidemiol, 159*(7), 683-692.

Miller, A. C., Blakely, W. F., Livengood, D., Whittaker, T., Xu, J., Ejnik, J. W., et al. (1998). Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. *Environ Health Perspect, 106*(8), 465-471.

Miller, A. C., Fuciarelli, A. F., Jackson, W. E., Ejnik, E. J., Emond, C., Strocko, S., et al. (1998). Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. *Mutagenesis, 13*(6), 643-648.

Miller, A. C., Mog, S., McKinney, L., Luo, L., Allen, J., Xu, J., et al. (2001). Neoplastic transformation of human osteoblast cells to the tumorigenic phenotype by heavy metal-tungsten alloy particles: induction of genotoxic effects. *Carcinogenesis, 22*(1), 115-125.

Miller, A. C., Xu, J., Stewart, M., Brooks, K., Hodge, S., Shi, L., et al. (2002). Observation of radiation-specific damage in human cells exposed to depleted uranium: dicentric frequency and neoplastic transformation as endpoints. *Radiat Prot Dosimetry, 99*(1-4), 275-278.

NATO. (2005). *NATO Handbook on the Medical Aspects of NBC Defensive Operations (Nuclear)* Retrieved. from.

North Atlantic Treaty Organization. (2005). *NATO Handbook on the Medical Aspects of*

NBC Defensive Operations (Nuclear) NATO/NSA.
Operation Iraq Freedom (OIF) Casualty Status. (2007). Retrieved November 15, 2007, from http://www.globalsecurity.org/military/library/news/2007/11/071108-casualty.pdf
Parham, D. M. (2001). Pathologic classification of rhabdomyosarcomas and correlations with molecular studies. *Mod Pathol, 14*(5), 506-514.
Partlow, J. (2007, February 13, 2007). Military ties Iran to arms in Iraq. *Washington Post,* p. A01.
Peyser, A., Khoury, A., & Liebergall, M. (2006). Shrapnel management. *J Am Acad Orthop Surg, 14*(10 Suppl), S66-70.
Rigdon, R. H. (1974). Plastics and carcinogenesis. *South Med J, 67*(12), 1459-1465.
Ritz, B., Morgenstern, H., Crawford, D., & Young, B. (2000). The effects of internal radiation exposure on cancer mortality in nuclear workers at Rocketdyne/Atomics International. *Environ Health Perspect, 108*(8), 743-751.
Rosenblatt, J. D., & Parry, D. J. (1992). Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. *J Appl Physiol, 73*(6), 2538-2543.
Schenck, N. L., & Kronman, B. S. (1977). Hoarseness and mass in the neck 30 years after pentrating shrapnel injury. *Ann Otol Rhinol Laryngol, 86*(2 pt. 1), 259.
Schwindt, P. C., Spain, W., & Crill, W. E. (1984). Epileptogenic action of tungstic acid gel on cat lumbar motoneurons. *Brain Res, 291*, 140-144.
Stevens, W., & Lang, P. (1994). *Improved tungsten penetrators*. Danbury, CT: Advanced Technologiy Materials.
Stokinger, H. E. (1981). Tungsten. In G. D. Clayton & F. e. Clayton (Eds.), *Pattys Industrial Hygiene and Toxicology* (pp. 1981-1995). New York: John Wiley and Sons.
Stromberg, B. V. (1990). Symptomatic lead toxicity secondary to retained shotgun pellets: case report. *J Trauma, 30*(3), 356-357.
Sunderman, F. W., Jr. (1989). Carcinogenicity of metal alloys in orthopedic prostheses: clinical and experimental studies. *Fundam Appl Toxicol, 13*(2), 205-216.
Symonds, R. P., Mackay, C., & Morley, P. (1985). The late effect of grenade fragments. *J R Army Med Corps, 131*(2), 68-69.
Terato, H., Tanaka, R., Nakaarai, Y., Furusawa, Y., & Ide, H. (2004). Analysis of DNA damage generated by high-energy particles. *Nucleic Acids Symp Ser (Oxf)*(48), 145-146.
Thomassen, M. J., Buoen, L. C., Brand, I., & Brand, K. G. (1978). Foreign-body tumorigenesis in mice: DNA synthesis in surface-attached cells during preneoplasia. *J Natl Cancer Inst, 61*(2), 359-363.
Thomassen, M. J., Buoen, L. C., & Brand, K. G. (1975). Foreign-body tumorigenesis: number, distribution, and cell density of preneoplastic clones. *J Natl Cancer Inst, 54*(1), 203-207.
USACHPPM. (2006). OIF DU bioassay and metal fragment analysis: AMEDD.
White, M. (2007). Iraq cooalition casualty count. Retrieved November 16, 2007, from http://icasualties.org/oif/
World Health Organization. (2003). Depleted Uranium. Retrieved November 16, 2007, from http://www.who.int/mediacentre/factsheets/fs257/en/
Zouris, J., Walker, G., Dye, J., & Galarmeau, M. (2006). Wounding patterns for U.S.

Marines and Sailors during Operation Iraqi Freedom, major combat phase. *Military Medicine, 171*(3), 246-252.

Treatment of Victims of Violence: From the Frontlines to City Streets

CAPT Roberta Proffitt Lavin

Uniformed Services University of the Health Sciences

Graduate School of Nursing

4301 Jones Bridge Road

Bethesda, Maryland 20814-4799

Co- Authors:

Christine E. Kasper, PhD, RN, FAAN

John F. Kalinich, PhD

Abstract

Gunshot wounds and shrapnel injuries are all too common during war, but even within the borders of the United State the rate of gun violence is alarmingly high. It is a widely accepted practice to remove embedded fragments that are easily accessible, but to leave those that are hard to reach. However, the debate over whether to aggressively remove embedded shrapnel is reemerging as a result of continued research into the health effects of embedded heavy metal fragments such as depleted uranium and tungsten alloy and concerns over carcinogenicity of some heavy metals. Careful evaluation of the literature and emerging research highlights the need to review the practice of leaving shrapnel embedded and identifies a gap in nursing research.

Nurses have always been critical to the care of those who put themselves in harms way. From the Crimean War to Operation Iraqi Freedom (OIF), nurses cared for the injured, improved practices and translated lessons learned into better care that benefited not only the military, but the civilian. Care of those with shrapnel and gunshot wounds (also referred to as ballistic injuries) bears similarity to the care of victims of gun violence and terrorism on our streets. What is learned in the combat zone may provide insight into civilian care and what can be evaluated from the civilian literature may provide insight into potential long-term complications of our war wounded, especially those with retained metal fragment that may be carcinogenic.

In this article the importance of understanding the current state of the science of retained fragments will become clear, as all nurses—and indeed most Americans—are concerned about the care of victims of violence whether it occurs in Iraq or on our city streets. Many victims of violence with injuries involving retained fragments leave the military before long-term complications become apparent. In fact, while there are a few articles on wound ballistics and immediate care of persons with gunshot and shrapnel wounds in the nursing literature, they do not address the debate over removal of embedded fragments, the likely long-term implications, or the differences between civilian and military injuries. This article concludes with a clarification of the critical role nurses must play in policy formulation related to the long-term care of victims of violence.

Background

Weapons can be divided into low, medium, and high velocity as well as explosive devices. These weapons are used in different types of violence. For example, the improvised explosive device (IED) is more commonly used in terrorist incidents, the high velocity weapon in war fighting, and the low velocity weapon in street violence (see Table 1) [1-4]. Likewise, different types of weapons generally cause injury to different locations of the body (see Table 2) [5, 6].

Advances in health care have resulted in improved survival rates for gunshot victims and combat casualties. The overall survival rate for penetrating injuries, including gunshot and shrapnel wounds, is dependent on many factors including number of injuries, location, time from injury to treatment, and Glascow Comma Scale. The state of Oklahoma, which tracks gun injuries and not just fatalities, reported an incidence of gunshot injuries of 56 per 100,000 in 1995 with an overall survival rate of 70% of which 41% were discharged home from the emergency

department [1]. Most gunshot victims who die from their wounds do so within the first seven days, whereas explosion victims may die later in their hospitalization [7].

In the civilian population deaths from firearms are believed to be a good indicator of firearms violence, despite the lack of nationwide data on nonfatal gunshot wounds. The rate of nonfatal gunshot wounds is estimated to be 2.6 times the rate of fatal gunshot wounds indicating the magnitude of this public health problem [8]. In 2004 there were 10.1 deaths per 100,000 related to firearms, which is an increase from the 1998 rate of 6.5 per 100,000 [9, 10]. Moreover, approximately 12% of civilian traumatic brain injuries (TBI) are from violence, including gunshot wounds, whereas in the military penetrating TBIs are approximately 50% of all military-related injuries; the majority come from bullets and fragments [11]. In the civilian population the majority of TBIs come from blunt force trauma.

It addition to injuries from gun violence in the United States, it has been estimated that over 28,000 Americans have been wounded in Iraq since March 2003 [5, 12]. Fragmentation wounds accounted for approximately 49% of wounds in the Persian Gulf War and 46% of wounds in the first phase of Operation Iraqi Freedom (OIF) [6, 13]. While a paucity of literature exists on the percentage of retained fragments from fragmentation wounds, Hamouda [14] found that one-third of patients had retained fragments, which, if predictive of gunshot and shrapnel wounds in general, would indicate that approximately 15% of those wounded will carry fragments in their bodies for the rest of their lives. Because the survival rate in combat trauma has improved to about 90% the number with retained fragments is notable [15].

It is not the general practice to remove retained fragments unless they pose a danger. The number of retained fragments is less likely to occur in the civilian population rather than the military for two reasons: 1) most civilian injuries are not part of a mass casualty event, because such events may require altered standards of care, and 2) most civilian injuries are from low-velocity gunshots and not explosive devices.

Approach to Treatment

The approach to treating patients with retained fragments remains controversial [16]. While some recommend removing fragments whenever possible others suggest a conservative approach - leaving the fragments in place [17-19]. The debate over approaches to treatment is largely based on the best immediate outcome for the patient, assuming that there are no or minimal long-term complications of embedded fragments. The most common

manifestation of a retained fragment is pain, but more serious complications include neurological symptoms, vascular compression, abscesses, granulomas, infections, lead synovitis and lead intoxication [17]. Additionally, modern test such as MRI can cause torque on the metal fragments and cause artifacts in the MRI image; this is more of an issue with wartime munitions than it is with civilian type gunshot wounds because of the characteristics of the retained fragments [20].

Fragment Identification

The basic principles of treatment of gunshot wounds remains largely unchanged since World War II [21]. The simplest method of identifying a retained fragment is to do a complete inspection of the body and count the number of entry and exit wounds, which should be equal [2, 22]. If the number is not equal, then there is a retained fragment. Counting entry and exit wounds may be less effective if the bullet hits a bone and fragments, further, this method is not effective with IEDs that result in a large number of wounds.

A second method for fragment identification is a total body digital x-ray, which is quick and can be done with the patient supine [23]. X-rays are especially helpful following a terrorist event with an IED. IEDs may contain metallic fragments that can be easily identified on x-rays, which will then help to determine where the fragments are lodged. If there are multiple retained fragments, then the ability to provide a precise location decreases [23]. Once fragments are identified, CT scanning may be needed to identify the path and location of the fragments [23]. When a fragment is identified a decision can be made on whether to treat conservatively or aggressively debride the area, including removing all fragments. However, in a mass casualty situation, it may not be possible to search for fragments due to the large number of casualties [17]. For example, the 2004 Madrid train bombings resulted in over 2000 casualties, thus treatment options were limited to the resources available [24].

Outcomes and Retained Fragments

The Army's manual on Emergency War Surgery appropriately notes that, "the more things change the more things stay the same"[25]. Shrapnel injuries under any circumstances can lead to a crisis, but when combined with embedded fragments that are potentially carcinogenic the risk of a crisis increases. A crisis is both an "emotional reaction to a hazardous event" and an opportunity for growth [26, p. 11]. It is logical that it is better to prevent a crisis than to respond to one once it has occurred. But like most complex issues in society it is not an either-or proposition.

As health care providers, it is imperative that we not only be prepared for and makes efforts to prevent a crisis, but also have the ability to respond.

In the majority of cases, gunshot wounds and shrapnel will have caused its damage at the time of the injury and the victims will suffer no further harm. However, in addition to causing skeletal muscle pathology, munitions composed of heavy metals, such as depleted uranium and tungsten alloys, have chemical properties that are also carcinogenic. There is ample evidence from the literature that shrapnel injuries, while infrequent, can continue to cause health complications decades after the initial injury (see Table 3). [27-32]. However, the risk may be changing as new metals are added to military munitions as well as to bullets and bird shot for civilian use.

There are few reported cases of adverse effects from embedded lead bullets in the literature; however, one reported finding is inflammation [33]. When lead remains embedded in a joint it is degraded through physical and chemical processes, which results in metal fragments spreading particles [34].

Case studies of persons with gunshot wounds with retained lead fragments have consistently shown significantly elevated blood lead levels for months and adverse health effects for decades [32, 35-37]. It is believed that a blood lead level > 30μg/dL, which is greater than 10 times the population mean, affects the nervous system and may impair recovery [33, 35].

As a result of friendly fire incidents, several soldiers from the first Gulf War have retained depleted uranium (DU) fragments. These veterans have persistently elevated levels of uranium in their urine although no other adverse health effects have been reported [38-40]. In experimental models, embedded DU has been shown to relocate and deposit in the kidneys and bone, as well as affect oncogene expression and decrease fertility [41]. DU also has both transforming [42] and mutagenic properties [43]. It has been shown that tumorigenic potential can be conferred on a human osteoblastic sarcoma cell line exposed to soluble and insoluble DU compounds [42].

Recently tungsten alloys have replaced lead in some small-caliber ammunition (so-called "green bullets" [44]) and DU in kinetic-energy penetrators [45, 46]. Despite its many uses, very little is known about the health effects of internalized tungsten. The majority of data come as a result of industrial exposures, particularly hard-metal disease, and biokinetic experiments using radioactive tungsten. Tungsten is rapidly absorbed (up to 25 times faster than DU) by the body, regardless of the route of exposure [47]. Once in the bloodstream, tungsten is distributed throughout the

body before being excreted via the kidney in the urine. The prevailing theory is that elemental tungsten or insoluble tungsten compounds have only limited toxicity [48-52].

Information on the health effects of embedded fragments of tungsten alloy is limited. Studies on tungsten-containing waterfowl shot showed no adverse health effects when embedded into the breast muscle of mallards [53]. A recent study investigating the health effects of a militarily-relevant tungsten alloy (92%W/5% Ni/3% Co) in a rodent model showed that, when embedded into the hind legs of rats, this particular tungsten alloy induced tumors in 100% of the animals [54]. The tumors were classified as rhabdomyosarcomas (RMS) and were highly aggressive, metastasizing to the lungs. However, there are no human reports on retained fragments containing tungsten alloy in humans.

The most comprehensive data available to date on retained fragments actually involves bone fragments from the Vietnam Head Injury Study. During Vietnam it was standard practice to remove as many of the fragments as possible even if it meant multiple surgeries [11]. Since then, follow-up studies have shown that retained bone does not significantly impact morbidity, mortality, or complications, thus supporting a conservative approach unless such complications require further surgery [11]. In fact, there are those that advocate no surgery unless life-threatening complications arise and recommend that no surgery may be better option resulting in fewer epileptogenic foci in traumatic brain injuries [18, 19]. The decision for surgery or no surgery in these cases is based on the size of the wound, amount of embedded material, and administration of antibiotics.

Marquardt [55] reports the longest latency in the literature concerning brain abscesses following a penetrating TBI due to shrapnel, but reviewed 10 other cases with long latency periods, demonstrating the rarity of the occurrence. He maintains that while the metal fragments may be sterile at the time of impact due to the heat they produce and that the bone fragments and subsequent procedures may result in bacterial invasion. Even so, it is widely a widely supported belief that there is no increased risk of infection from retained fragments and further surgery may actually increase the risk of neurological deficit [56-60]. However, Gliddon (61) found that 67% of those with retained fragments developed seizures; other studies also supported Gliddon's findings [62, 63]. The debate continues over whether there is a difference in outcomes with retained bone and metallic fragments. Other than TBI and elevated lead levels, there is little literature and follow-up related to long-term health consequences of retained

fragments.

Reasons for Concern

When a policy or health care practice becomes "black-boxed", health care professionals may become complacent in evaluating treatment options based on advances in care and technology. What is interesting is that the decision to leave fragments embedded appears to be based on retrospective review of the literature, patient records, and the belief that retained fragments cause no harm. Even with lead poisoning—a condition that has had public health attention—cases are not identified until the patient is extremely symptomatic due to a lack of routine follow-up [64]. There is little discussion of the composition of the fragments that caused problems and virtually no long-term follow-up of those with the retained fragments in skeletal muscle, which is critical since it has been estimated that 60 – 70% of all wounds result in musculoskeletal injuries [65].

It is generally recommended that civilian and military ballistic injuries be treated the same [8]. However, there are special concerns for those who have been in a war zone. The findings of Kalinich et al. [54] raise serious concerns about the health effects of tungsten/nickel/cobalt alloys in munitions. Shrapnel is generally not removed unless it is either a large fragment or in a location where it is likely known to cause further damage [65]. In response to the findings reported by Kalinich et al. [54], some metal fragments were removed from U.S. soldiers in Iraq to determine if they contained HMTA. As of September 2006, approximately 1900 individuals had been screened by bioassay and approximately 2100 specimens had been analyzed. Analysis of 68 fragments from 60 individuals from over 28,000 wounded soldiers showed that none contained tungsten alloy [66, 67]; three fragments from two individuals did contain DU.

The studies on tungsten alloy have yet to address the removal of shrapnel on muscle function. Given the apparent lethal toxicity of tungsten alloy, it is imperative to review the standard practices for fragment identification and removal. Further, it is advisable to provide regular follow-up to those that have been exposed to heavy metals that may be carcinogenic. Shrapnel injuries not involving tungsten alloy can also result in tumor formation and other long-term health consequences [27-31]. Thus, future research should focus on two areas: 1) the assessment of the health effects of embedded fragments, especially the unique materials found on today's battlefields, and 2) the *in situ* identification of shrapnel using blood or urine biomarkers. Given that information, one can make a more informed

decision whether to remove fragments or leave them in place.

The potential expanded use of heavy metals in munitions and the introduction of new materials, such as explosively-shaped charges on the battlefield, increase the risk of shrapnel wounds in both combatants and noncombatants [68]. Knowledge of the long-term consequences of gunshot and shrapnel injuries is now an issue for both military and civilian health care with timely and proper care is critical for those wounded. Nurses must carefully monitor patients with these injuries and be aware of the potential long-term health consequences that fragments present. It is time to reexamine the topic of fragment removal and educate health care professionals on the potential long-term health consequences of embedded fragments, develop longitudinal studies that follow those persons with embedded fragments, and explore policy options that ensure that victims of violence are not injured twice.

The views expressed in this article are those of the author and do not necessarily reflect those of the U.S. Department of Health and Human Services or the Uniformed Services University of the Health Sciences. There is no conflict of interest in this publication. (Comment of any funding)

Table 1. Percentage of wounds by weapon type and population

	Civilian (1, 33)	Military (2-4, 15)
Low-velocity (handguns)	76 - 97	
Medium-velocity (shotguns)	3 - 12	
High-velocity (rifles)	1 - 12	28 - 55 %
Improvised Explosive Device (largely shrapnel)	0	45 - 72 %

Table 2. Percentage of wounds by location in terrorist incidents (4, 7)

	Gunshot	Explosion
Head	21	35
Trunk	39	27
Upper extremities	19	19
Lower extremities	21	19

Table 3. Case report and study characteristics

Reference	(n)	Injury and Initial Treatment	Outcome	Year after Injury
Shroyer et al. (34)	1	Retained lead bullet in spinal cord; loss of muscle strength in legs and back; cyst surrounding bullet	Removed; recovered	10 years
Wakabayashi et al. (69)	1	2 cm breast mass (noncancerous) with a 0.5 mm metallic bullet particles inside with others in surrounding area	Recovered	20 years
Wellington (70)	1	Pyopneumothorax and encysted empyema developed around a FB	FB removed; recovered	17 years
Peral-Gutierrez de Ceballos et al. (24)	89	Madrid train bombings--89 with shrapnel injuries; wound debridement accounting for 33% of the surgeries in first 24 hours	Debridement; 23 critical – actual outcomes not reported	Same day
Marquardt et al. (55)	1	Seizures 3 yrs post-injury; rapidly progressing headaches, weakness on right side, speech difficulty	Removed; improvement in brain profusion and focal dysrhythmia.	10 and 52 years
Aarabi et al. (71)	200	Unprovoked posttraumatic epilepsy	67% of those with retained fragments developed seizures	6 – 154 months
Fildes et al. (72) (literature review)	26	Retained fragments, bullets, and pellets resulting in urethral obstruction and renal colic	8 were passed, 16 required surgery, 1 cystoscopy, 1 percutaneous nephrostomy	1 week – 26 years
Knox et al. (29)	1	Expanding painful swelling over left lower chest	Surgical drainage and 10 x 9 mm piece of shrapnel removed	62 years
Eylon et al. (73)	4	All presented with pain; yellowish-greenish or black-green fluid filled mass surrounding shrapnel; 1 inflammatory reaction	Removed	9 – 36 years
Symbas et al. (74)	225	Shrapnel, bullets, pellets in the heart; all presented with and without symptoms; e.g., chest pain, infection, arrhythmia, cardiac neurosis, and cerebral vascular accident	104 removed; 20 attempted to remove or left embedded	Most within 2 day of injury
Gasparovic et al. (16)	1	Pediatric poly trauma patient with retained intracardiac shrapnel; no cardiac symptoms and other more critical injuries	Left in place	Not noted
Singer et al. (75)	1	3 bullets left embedded and after 2 weeks developed fever, leukocytosis and left lower lobe necrosis; bullet migrated	Removed; resolved	2 weeks
Veselko et al. (17)	2	Proximity to nerve; pain on movement of shoulder	Removed; resolved	No data
Shurbaji et al. (18)	1	Head wound through helmet; infection and seizures developed	Removed; debrided	3 days
Manganas et al. (76)	1	Massive hemoptysis from a pulmonary arteriovenous malformation	Double lumen endobronchial intubation, bronchial arterigraphy and embolization, and right lower lobectomy	30 years
Dickson et al. (8)	2	Pain	Removed under local anesthesia	Not noted

Scartozzi et al. (77)	1	Recurrent infections of foot	Removed; resolved	19 years
Shen & Hirschtick (78)	1	Bullet fragments in synovium; Back and abdominal pain, nausea, and constipation; increased lead level; returned in 5 months with increased lead level	EDTA & dimercaprol; lead level decreased, symptoms reolved; after repeated treatment in 5 months recommended surgery	6 years
Nguyen et al. (64)	120	Prospective cross-sectional study of persons with retained lead fragments; 5 had elevated lead levels, admitted other exposures	Follow-up	2 – 336 months
Selva-O'Callaghan et al. (79)	1	Recurrent abdominal pain	ETDA, pellets removed; resolved	4 years
Coon et al. (80)	1	Recurrent abdominal pain, vomiting, & anorexia; decreased growth & school performance; lead level taken on 3rd hospitalization	Succimer chelation; removed; resolved	2 years
Porter et al. (81)	1	Routine prenatal ultrasound revealed multiple abnormalities; child born with multiple birth defects and elevated lead level	Chelation therapy for both; surgical debulking of fragments from mother; lead level dropped	15 years
McQuirter et al. (33)	502	Tracked persons with fragments; 38.1% had elevated lead levels at 3 months and 2.1 % at 24 months	No information provided	24 months

Table 4. Complications and treatment of retained fragments

Location	Potential Problems	Recommendations
Craniocerebral	Bleeding Migration Infection	Primary debridement & wound closure for GCS 13-15 with limited tissue destruction Primary closure without active bleeding (82) Remove shrapnel with documented migration, large objects that are accessible or pose potential risk Consider endovascular therapy as first-line option if object crosses 2 dural compartments or involves facial or orbital regions (83)
Spinal Canal	Chronic infection Bone overgrowth Spinal cord compression (34, 84)	Remove FB > 1 cm (70) Careful histories and screening mammograms (69) Removal not recommended except in cauda equine injury (85)
Heart	Pericardium or pericardial space - pericarditis (74) Free or protruding into the cavity –endocarditis	Fragments in the myocardium, pericardium & pericardial space - leave in place Not completely embedded in the myocardium -remove Intracavitary spaces especially on the right side - monitor for movement into the pulmonary artery from where they can be removed Intracavitary or partially embedded in the mayocardium that are found late - follow to determine if they are encapsulated, if so they can be followed Large, symptomatic, or those with irregular margins especially if located next to an artery - remove (74) Asymptomatic with intracardiac fragment – manage conservatively with regular follow-up examination, but any complication require surgical intervention (16)
Pulmonary artery	Signs the fragment embolizes: pulmonary infarction, erosion or sepsis, asymptomatic (74, 86)	Some suggest observation only, others catheter extraction or thoracotomy (75, 87, 88) Removal of bullet emboli to the pulmonary artery due to risk of pulmonary complications (75) Asymptomatic – monitor (74, 86)
Abdomen	Renal colic (72)	Note GSW in history
Musculoskeletal	Joint damage; pain in weight bearing area; arthritis	Debride as much shrapnel as possible Remove fragment in joints (64) Remove devitalized tissue (89) Fragment not in joints may remain (64) Sequential x-rays
General	Damage to neurovascular structures; plubism	Remove fragment Surveillance every 3 months for 1 year Chelation therapy (33)

References

1. **Archer P.** Injury update: A report to Oklahoma injury surveillance participants. 1997.
2. **Folio L, McHugh C, Hoffman MJ.** The even-number guide and imaging ballistic injuries. *Radiol Technol.* 2007;78(3):197-203.
3. **Gofrit ON, Kovalski N, Leibovici D, Shemer J, O'Hana A, Shapira SC.** Accurate anatomical location of war injuries: analysis of the Lebanon war fatal casualties and the proposition of new principles for the design of military personal armour system. *Injury.* 1996;27(8):577-81.
4. **Peric M, Hamel D, Rac S, et al.** Civilian medicine and war: transformation of a health centre to a war hospital in Croatia (July 1991-October 1992). *Injury.* 1996;27(6):395-9.
5. Operation Iraq Freedom (OIF) Casualty Status. 2007.
6. **Burkle F, Newland C, Meister S, Blood C.** Emergency medicine in the Persian Gulf War Part 3: Battlefield casualties. *Annals of Emergency Medicine.* 1994;23(4):755-760.
7. **Peleg K, Aharonson-Daniel L, Stein M, et al.** Gunshot and explosion injuries: characteristics, outcomes, and implications for care of terror-related injuries in Israel. *Ann Surg.* 2004;239(3):311-8.
8. **Dickson K, Watson TS, Haddad C, Jenne J, Harris M.** Outpatient management of low-velocity gunshot-induced fractures. *Orthopedics.* 2001;24(10):951-4.
9. **U.S. Department of Health and Human Services.** Healthy People 2010: Understanding and improving health. In: 2nd, ed. Washington, DC: U.S. government Printing Office; 2000.
10. **National Center for Health Statistics.** All injuries. Hyattsville: U.S. Department of Health and Human Services; 2007.
11. **Zasler N, Serio CD.** Traumatic brain injury. In: Zajtchuk R, ed. *Rehabilitation of the Injured Combatant.* Vol. 1. Washington, DC: Borden Institute, Office of the Surgeon General.
12. **White M.** Iraq cooalition casualty count. 2007.
13. **Zouris J, Walker G, Dye J, Galarmeau M.** Wounding patterns for U.S. Marines and Sailors during Operation Iraqi Freedom, major combat phase. *Military Medicine.* 2006;171(3):246-252.
14. **Hamouda H, Witse E, Moghani N, Shahwan A, Nygaard O.** Soft tissue infection after missile injuries to the extremities - A non-randomized, prospective study in Gaza City. *Prehospital and Disaster Medicine.* 2007;22(2):106-108.
15. **Clark ME, Bair MJ, Buckenmaier CC, Gironda RJ, Walker RL.** Pain and OIF/OEF combat injuries: Implications for research and practice.
16. **Gasparovic H, Stern-Padovan R, Batinica S, Saric D, Jelic I.** Intracardiac shrapnel in a polytraumatized child. *Ann Thorac Surg.* 2004;77(3):1083-5.
17. **Veselko M, Trobec R.** Intraoperative localization of retained metallic fragments in missile wounds. *J Trauma.* 2000;49(6):1052-8.
18. **Shurbaji A, Rosahl SK, Feigl GC, Samii M.** Metallic foreign body in the cavernous sinus causing delayed formation of brain abscess after missile injury. *J Trauma.* 2006;60(5):1135-7; discussion 1137.
19. **Amirjamshidi A, Abbassioun K, Rahmat H.** Minimal debridement or simple wound closure as the only surgical treatment in war victims with low-velocity penetrating head injuries. Indications and management protocol based upon more than 8 years follow-up of 99 cases from Iran-Iraq conflict. *Surg Neurol.* 2003;60(2):105-10; discussion 110-1.
20. **Teitelbaum GP, Yee CA, Van Horn DD, Kim HS, Colletti PM.** Metallic ballistic fragments: MR imaging safety and artifacts. *Radiology.* 1990;175(3):855-9.
21. **Secer HI, Daneyemez M, Gonul E, Izci Y.** Surgical repair of ulnar nerve lesions caused by gunshot and shrapnel: results in 407 lesions. *J Neurosurg.* 2007;107(4):776-83.
22. **Blank-Reid C, Reid PC.** Penetrating trauma to the head. *Crit Care Nurs Clin North Am.* 2000;12(4):477-87.
23. **Sosna J, Sella T, Shaham D, et al.** Facing the new threats of terrorism: radiologists' perspectives based on experience in Israel. *Radiology.* 2005;237(1):28-36.
24. **de Ceballos JP, Turegano-Fuentes F, Perez-Diaz D, Sanz-Sanchez M, Martin-Llorente C, Guerrero-Sanz JE.** 11 March 2004: The terrorist bomb explosions in Madrid, Spain--an analysis of the logistics, injuries sustained and clinical management of casualties treated at the closest hospital. *Crit Care.* 2005;9(1):104-11.
25. **Lounsbury D.** Emergency War Surgery. 3rd ed. Washington, DC: Borden Institute; 2004.

26. **Infante MS.** *Crisis theory: A framework for nursing practice* Reston, VA: Reston Publishing Company; 1982.
27. **Lindeman G, McKay MJ, Taubman KL, Bilous AM.** Malignant fibrous histiocytoma developing in bone 44 years after shrapnel trauma. *Cancer.* 1990;66(10):2229-32.
28. **Schenck NL, Kronman BS.** Hoarseness and mass in the neck 30 years after pentrating shrapnel injury. *Ann Otol Rhinol Laryngol.* 1977;86(2 pt. 1):259.
29. **Knox J, Wilkinson A.** Shrapnel presenting with symptoms 62 years after wounding. *Br Med J (Clin Res Ed).* 1981;283(6285):193.
30. **Ligtenstein DA, Krijnen JL, Jansen BR, Eulderink F.** Forgotten injury: a late benign complication of an unremoved shrapnel fragment--case report. *J Trauma.* 1994;36(4):580-2.
31. **Symonds RP, Mackay C, Morley P.** The late effect of grenade fragments. *J R Army Med Corps.* 1985;131(2):68-9.
32. **Dillman RO, Crumb CK, Lidsky MJ.** Lead poisoning from a gunshot wound. Report of a case and review of the literature. *Am J Med.* 1979;66(3):509-14.
33. **McQuirter JL, Rothenberg SJ, Dinkins GA, Kondrashov V, Manalo M, Todd AC.** Change in blood lead concentration up to 1 year after a gunshot wound with a retained bullet. *Am J Epidemiol.* 2004;159(7):683-92.
34. **Shroyer RN, Fortson CH, Theodotou CB.** Delayed neurological sequelae of a retained foreign body (lead bullet) in the intervertebral disc space. *J Bone Joint Surg Am.* 1960;42-A:595-9.
35. **Gerhardsson L, Dahlin L, Knebel R, Schutz A.** Blood lead concentration after a shotgun accident. *Environ Health Perspect.* 2002;110(1):115-7.
36. **Magos L.** Lead poisoning from retained lead projectiles. A critical review of case reports. *Hum Exp Toxicol.* 1994;13(11):735-42.
37. **Stromberg BV.** Symptomatic lead toxicity secondary to retained shotgun pellets: case report. *J Trauma.* 1990;30(3):356-7.
38. **McDiarmid MA, Engelhardt S, Oliver M, et al.** Health effects of depleted uranium on exposed Gulf War veterans: a 10-year follow-up. *J Toxicol Environ Health A.* 2004;67(4):277-96.
39. **McDiarmid MA, Keogh JP, Hooper FJ, et al.** Health effects of depleted uranium on exposed Gulf War veterans. *Environ Res.* 2000;82(2):168-80.
40. **McDiarmid MA, Squibb K, Engelhardt S, et al.** Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. *J Occup Environ Med.* 2001;43(12):991-1000.
41. **NATO.** NATO Handbook on the Medical Aspects of NBC Defensive Operations (Nuclear) In: NATO, ed. Vol. 1: NATO/NSA; 2005.
42. **Miller AC, Blakely WF, Livengood D, et al.** Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. *Environ Health Perspect.* 1998;106(8):465-71.
43. **Miller AC, Fuciarelli AF, Jackson WE, et al.** Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. *Mutagenesis.* 1998;13(6):643-8.
44. **Bogard JS, Yuracko KL, Murray ME, Lowden RA, Vaughn NL.** (Oak Ridge National Lab). Application of life cycle analysis: the case of green bullets. 1988. Report No.: ORNL/CP-98265.
45. **Kerley CR, Easterly CE, Eckerman KF, et al.** (Oak Ridge National Labs). Environmental acceptability of high-performance alternatives for depleted uranium penetrators. 1996. Report No.: ORNL/TM-13286.
46. **Stevens W, Lang P.** (Advanced Technologly Materials). Improved tungsten penetrators. 1994.
47. **Leggett RW.** A model of the distribution and retention of tungsten in the human body. *Sci Total Environ.* 1997;206:147-165.
48. **Kazantzis G.** Tungsten. In: Friberg L, Nordberg FF, Vouk VB, eds. *Handbook on the Toxicology of Metals.* New York: Elsiver; 1986:610-622.
49. **Lauring L, Wergeland FL.** Ocular toxicity of newer industrial metals. *Military Medicine.* 1970;135:1171-1174.
50. **Stokinger HE.** Tungsten. In: Clayton GD, Clayton Fe, eds. *Pattys Industrial Hygiene and Toxicology.* New York: John Wiley and Sons; 1981:1981-1995.
51. **Kerwien SC.** (U.S. Army Armament Research, Development and Engineering Center, Picatinny Arsenal). Toxicity of tungsten, mlybdenum, and tantalum and the environmental and occupational laws associated with their manufacutre, use, and disposal. 1996. Report No.: ARAED SP-96002.

52. **Agency for Toxic Substances and Disease Registry.** Draft toxicological profile for tungsten. In: Prevention CfDCa, ed; 2003.
53. **Kraabel BJ, Miller MW, Getzy DM, Ringelman JK.** Effects of embedded tungsten-bismuth-tin shot and steel shot on mallards. *J Wildlife Dis.* 1996;32:1-8.
54. **Kalinich JF, Emond CA, Dalton TK, et al.** Embedded weapons-grade tungsten alloy shrapnel rapidly induces metastatic high-grade rhabdomyosarcomas in F344 rats. *Environ Health Perspect.* 2005;113(6):729-34.
55. **Marquardt G, Schick U, Moller-Hartmann W.** Brain abscess decades after a penetrating shrapnel injury. *Br J Neurosurg.* 2000;14(3):246-8.
56. **Chaudhri KA, Choudhury AR, al Moutaery KR, Cybulski GR.** Penetrating craniocerebral shrapnel injuries during "Operation Desert Storm": early results of a conservative surgical treatment. *Acta Neurochir (Wien).* 1994;126(2-4):120-3.
57. **Gonul E, Baysefer A, Kahraman S, et al.** Causes of infections and management results in penetrating craniocerebral injuries. *Neurosurg Rev.* 1997;20(3):177-81.
58. **Aarabi B.** Causes of infections in penetrating head wounds in the Iran-Iraq War. *Neurosurgery.* 1989;25(6):923-6.
59. **Carey ME, Young H, Mathis JL, Forsythe J.** A bacteriological study of craniocerebral missile wounds from Vietnam. *J Neurosurg.* 1971;34(2 Pt 1):145-54.
60. **Hammon WM.** Retained intracranial bone fragments: analysis of 42 patients. *J Neurosurg.* 1971;34(2 Pt 1):142-4.
61. **Gliddon WO.** Gunshot wounds of the head: a review of the after-effects in 500 Canadian Pensioners from the Great War, 1914-1918. *Can Med Assoc J.* 1943;49:373-377.
62. **Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD.** Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. *Neurology.* 1985;35(10):1406-14.
63. **Weiss GH, Salazar AM, Vance SC, Grafman JH, Jabbari B.** Predicting posttraumatic epilepsy in penetrating head injury. *Arch Neurol.* 1986;43(8):771-3.
64. **Nguyen A, Schaider JJ, Manzanares M, Hanaki R, Rydman RJ, Bokhari F.** Elevation of blood lead levels in emergency department patients with extra-articular retained missiles. *J Trauma.* 2005;58(2):289-99.
65. **Peyser A, Khoury A, Liebergall M.** Shrapnel management. *J Am Acad Orthop Surg.* 2006;14(10 Suppl):S66-70.
66. **DoD.** Fact sheet: Tungsten/nickel/cobalt alloy study. In: Defense Do, ed; 2005.
67. **USACHPPM.** OIF DU bioassay and metal fragment analysis. AMEDD; 2006.
68. **Partlow J.** Military ties Iran to arms in Iraq. *Washington Post* 2007 February 13, 2007;A01.
69. **Wakabayashi M, Reid JD, Bhattacharjee M.** Foreign body granuloma caused by prior gunshot wound mimicking malignant breast mass. *AJR Am J Roentgenol.* 1999;173(2):321-2.
70. **Wellington JL.** The shrapnel awakes: pyopneumothorax and chronic empyema resulting from a foreign body retained for 17 years. *Can Med Assoc J.* 1962;87:349-51.
71. **Aarabi B, Taghipour M, Haghnegahdar A, Farokhi M, Mobley L.** Prognostic factors in the occurrence of posttraumatic epilepsy after penetrating head injury suffered during military service. *Neurosurg Focus.* 2000;8(1):e1.
72. **Fildes JJ, Betlej TM, Barrett JA.** Buckshot colic: case report and review of the literature. *J Trauma.* 1995;39(6):1181-4.
73. **Eylon S, Mosheiff R, Liebergall M, Wolf E, Brocke L, Peyser A.** Delayed reaction to shrapnel retained in soft tissue. *Injury.* 2005;36(2):275-81.
74. **Symbas PN, Picone AL, Hatcher CR, Vlasis-Hale SE.** Cardiac missiles. A review of the literature and personal experience. *Ann Surg.* 1990;211(5):639-47; discussion 647-8.
75. **Singer RL, Dangleben DA, Salim A, et al.** Missile embolism to the pulmonary artery: case report and pitfalls of management. *Ann Thorac Surg.* 2003;76(5):1722-5.
76. **Manganas C, Iliopoulos J, Pang L, Grant PW.** Traumatic pulmonary arteriovenous malformation presenting with massive hemoptysis 30 years after penetrating chest injury. *Ann Thorac Surg.* 2003;76(3):942-4.
77. **Scartozzi G, Hoffman L.** Chronic high velocity projectile injury to the foot. *J Am Podiatr Med Assoc.* 1989;79(5):236-41.

78. **Shen J, Hirschtick R.** Images in clinical medicine. Getting the lead out. *N Engl J Med.* 2004;351(19):1996.
79. **Selva-O'Callaghan A, Gomez-Acha J, Munne P, Vilardell-Tarres M.** A 21-year-old girl with recurrent abdominal pain after a robbery. *Lancet.* 2005;366(9491):1136.
80. **Coon T, Miller M, Shirazi F, Sullivan J.** Lead toxicity in a 14-year-old female with retained bullet fragments. *Pediatrics.* 2006;117(1):227-30.
81. **Porter S, Hanley E, Sing RF.** Fetal anomalies resulting from maternal bullet-related plumbism. *J Trauma.* 2004;56(6):1353-5.
82. **Izci Y, Kayali H, Daneyemez M, Koksel T, Cerrahoglu K.** The clinical, radiological and surgical characteristics of supratentorial penetrating craniocerebral injuries: a retrospective clinical study. *Tohoku J Exp Med.* 2003;201(1):39-46.
83. **Aschkenasy-Steuer G, Shamir M, Rivkind A, et al.** Clinical review: the Israeli experience: conventional terrorism and critical care. *Crit Care.* 2005;9(5):490-9.
84. **Schneider RC, Kahn EA.** Chronic neurological sequelae of acute trauma to the spine and spinal cord. IV. Problems pertaining to the treatment of retained foreign bodies. *J Bone Joint Surg Am.* 1959;41-A(3):457-62.
85. **Bhatoe HS, Singh P.** Missile injuries of the spine. *Neurol India.* 2003;51(4):507-11.
86. **Seidal T, Kindblom LG, Angervall L.** Rhabdomyosarcoma in middle-aged and elderly individuals. *Apmis.* 1989;97(3):236-48.
87. **Kortbeek JB, Clark JA, Carraway RC.** Conservative management of a pulmonary artery bullet embolism: case report and review of the literature. *J Trauma.* 1992;33(6):906-8.
88. **Bernini CO, Junqueira AR, Jr., Horita LT, Birolini D, Branco PD, de Oliveira MR.** Pulmonary embolism from gunshot missiles. *Surg Gynecol Obstet.* 1983;156(5):615-9.
89. **Bowyer G.** Debridement of extremity war wounds. *J Am Acad Orthop Surg.* 2006;14(10 Suppl):S52-6.

Dear Editors and Reviewers,

The attached article is the result of ongoing work on the effects of embedded tungsten alloy. The work builds on the a previous study by John Kalinich. The expanded use of heavy metals in munitions and the introduction of new materials, such as explosively-shaped charges, on the battlefield increase the risk of shrapnel wounds in both combatants and noncombatants. Knowledge of the long-term consequences of gunshot and shrapnel injuries is now an issue for both military and civilian health care and timely and proper care is critical for those wounded. In particular, the recent report that a military-grade tungsten alloy induced aggressive rhabdomyosarcomas (RMS) when implanted in the leg muscles of laboratory rats raises serious questions as to the timing of skeletal muscle changes that lead to neoplastic transformation.

Thank you for taking the time to review this article.

Best Regards,

Roberta Lavin

Carcinogenicity of Embedded Tungsten Alloy in Rodents

Tungsten Alloy

Roberta P. Lavin, MSN, APRN, BC

Uniformed Services University for the Health Sciences

Graduate School of Nursing

4301 Jones Bridge Road

Bethesda, Maryland 20814-4799

John F. Kalinich, PhD

Maxim Parekev, MD

Christine, E. Kasper, PhD, RN, FAAN

Total Number of Text Pages: 22

Total Number of Tables: 1

Total Number of Illustrations: 5

Acknowledgements: [Insert sources of support that require acknowledgment]

The views expressed in this article are those of the author and do not necessarily reflect those of the U.S. Department of Health and Human Services or the Uniformed Services University of the Health Sciences. There is no conflict of interest in this publication. (Comment of any funding)

Background: Throughout history tungsten and tungsten-containing materials have been used in a variety of applications. Recently these types of materials have be proposed as replacements for lead in small caliber ammunition and for depleted uranium in armor-penetrating munitions. Unfortunately, little if anything is known about the health effects of such materials when embedded intramuscularly, such as what will occur in a shrapnel wound. This study addresses some of those issues.

Methods: F344 rats were implanted intramuscularly with pellets (1 mm x 2 mm) of tantalum, nickel, or munitions-grade tungsten alloy containing tungsten/nickel/cobalt. Muscle samples were obtained at 1, 3, and 6 months postimplantation and assessed for a variety histologic changes and presence of apoptosis.

Results: No tumors developed in the tantalum-implanted (negative control) animals and no indications of neoplastic changes were seen around the implanted pellet. 100% of the Ni-implanted rats (positive control) developed large tumors by 6 months as did both groups (low dose and high dose) of tungsten alloy-implanted rats. Tumor development was slower in the tungsten alloy low-dose group. Implantation with tungsten alloy pellets also resulted in increased vascularity, high mitotic cell number, and both apoptosis and necrosis of the muscle.

Conclusions: This study while not definitive, suggests that regular monitoring of those individuals with shrapnel injuries containing heavy metals may be warranted.

Keywords: Car

Tungsten and its alloys (WA) are used in many everyday items such as light bulb filaments and x-ray tubes. It is also one of the metals increasingly used in military munitions as a replacement for lead and depleted uranium (DU) (1, 2). Beginning in the 1950's the U.S. began using tungsten carbide as a kinetic-energy armor piercing munitions, but as better armor was developed changes in munitions were required. Around 1972, a product containing 97.5% tungsten and 2.5% binder tungsten alloy that had a density of 18.5 gm/cc was used (1). Today, the most widely used WA munitions are comprised of 91-93% tungsten, 3-5% nickel, and either 2-4% cobalt or iron (4). Munitions containing tungsten may be in the form of penetrators for heavy armor, fragmentation warheads, small caliber ammunition, and even high performance lead-free shot for waterfowl hunting (2). The expanding use of these munitions is of great concern for two reasons: 1) when kinetic energy penetrators, like WA, pass through armor they tend to fragment causing shrapnel injuries, and 2) multiple countries in addition to the U.S., are using WA in munitions.

Elemental tungsten is a naturally occurring substance that most people in the U.S. population ingest through food, water, and air, resulting in background tungsten levels of 1-6 μg/L in blood and 0.085 μg/L in urine (3). Blood and urine levels can be used as biomarkers for exposure to tungsten and tungsten compounds. Although the risk is considered minimal, there is some evidence that occupational exposure to tungsten carbide and tungsten carbide with cobalt dust produced by hard metal industry may result in adverse health effects. However, the general belief is that the adverse effects are related to cobalt and

not tungsten (3). Associated illnesses include lung disease (pulmonary fibrosis and cancer) and neurological symptoms (memory and sensory deficits)(3). The Agency for Toxic Substance and Disease Registry (ATSDR) (3) concluded that tungsten oxide might contribute to pulmonary fibrosis because it has been shown to be able to generate hydroxyl radicals in an *in vitro* model system using human lung cells. Moreover, WA and other heavy metals have been shown in a rat model to cause malignant aggressive rhabdomyosarcoma (2).

Materials and Methods

This research was conducted under an Institutional Animal Care and Use Committee (IACUC) approved protocol and all rats were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life 4). The methods for this study have been previously published by Kalinich et al. (2) and are therefore only briefly described in this section.

Fischer 344 male rats (Harlan, Frederick, MD, 6 weeks of age) were used for implantation. Upon receipt, rats were screened for common rodent pathogens and were randomly assigned to groups. A negative control group (n=12) was implanted with tantalum (Ta) pellets, a positive control group (n=6) was implanted with nickel (Ni) pellets, and two experimental groups were implanted with either a high dose (20 pellets, n=17) or low dose (4 pellets, n=18) of WA pellets. The number and dimensions of the pellets (WA, Ni, and Ta) was

based on research previously conducted at AFRRI (5). Each rat received 20 pellets. Those in the low-dose WA group were implanted with 16 Ta pellets along with the 4 WA pellets. Thus metal loads across all groups were identical. The pellets were cylinders 1 mm in diameter x 2 mm in length. WA pellets consisting of 91.1% tungsten, 6% nickel, and 2.9% cobalt, similar to the tungsten alloy used in the U.S. tungsten-based kinetic energy penetrators, were purchased from Aerojet Ordnance Tennessee (Jonesborough, TN, USA). Tantalum (99.95% Ta) and nickel pellets (99.99 % Ni) were purchased from Alfa Aesar (Ward Hill, MA, USA). Tantalum was chosen as the implantation control metal because it is biologically inert (6) with a mass similar to tungsten, and it is used frequently in human prostheses (10, 11). Rats were euthanized at 1, 3 and 6 months and muscle and tumor samples collected.

Rats were fed a certified NTP-2000 diet (Quality Lab Products, Elkridge, MD, USA) to prevent excessive weight gain and to enhance longevity (13, 14). Acidified water (pH 2.5-2.8, using HCl) was provided ad libitum. Rats were paired housed in microisolator cages and were on a 12-hour light/dark cycle, with no twilight.

Surgery. The rats were implanted at approximately 9 weeks of age. Prior to surgery, all pellets were cleaned and chemically sterilized. Pellets were implanted in each gastrocnemius muscle spaced approximately 1.5 mm apart on the lateral side of each leg. Rats were closely monitored following surgery until they were ambulatory. An analgesic (buprenorphine hydrochloride, Reckitt and Colman, Hull, UK) was administered preoperatively and then as needed

postoperatively. Surgical sites were examined for signs of inflammation, infection, and local metal-induced toxicity daily for 2 weeks following surgery and weekly thereafter for the duration of the study.

Histopathology. Tissue samples from the gastrocnemius muscle were taken for histopathology examination. Gastrocnemius muscle and tumors were fixed in buffered formalin, processed and embedded in paraffin and sectioned on a microtome at 5-6 μm, mounted, and stained with hematoxylin and eosin (H&E) and gomori trichrome. Disintegration of myofibers, change in vasularity, tumor size, number of mitotic figures, and areas of necrosis were determined microscopically using a Nikon Optiphot fluorescence microscope. Images were taken and processed with NIS-Elements AR 2.3 package and digital images analyzed using ImageJ.

Apoptosis Staining. Apoptosis was quantified by using a TUNEL assay (ApopTag Red *In Situ,* Apoptosis Detection Kit, Chemicon Intl, USA) detecting single and double-strand breaks in deoxyribonucleic acid (DNA) that are associated with apoptosis using terminal deoxynucleotidyl transferase (TdT) labeling, which was carried out according to the manufacturer's instructions. Briefly, tissue was deparaffinized and pretreated with Proteinase K (20μg/mL) for 15 minutes at room temperature of 22.2 °C. Equilibration buffer was applied directly to the specimen for 10 seconds at room temperature followed by TdT enzyme (55 μL/5 cm^2) and incubation in a humidified chamber at 37^oC for 1 hour. Stop wash buffer was applied and after 15 minutes, rhodamine was applied and the slide incubated in a darkened humidified chamber for 30 minutes at room

temperature. Samples were washed in PBS and apoptotic cells were determined by counting the ApopTag red positive cells using fluorescence microscopy in 3 consecutive areas, containing approximately 100 cells per sample.

Results

The aim of this study was to assess the rate and magnitude of the development of rhabdomyosarcoma in rats implanted with WA, as well as the effects of WA on the surrounding muscle (Figure 1). We studied muscle samples from 53 rats that had been surgically implanted with metals (WA, Ta, or Ni) in their gastrocnemius muscle. Gastrocnemius muscle and tumor samples were taken from all rats at each time point with the exception of the Ni-implanted group which only had samples at 6 months post-implantation. The Fischer 344 rat was used for this study as they are frequently used in cancer research, toxicology studies, the National Toxicology Program's Carcinogen Bioassay Program, and the National Institute on Aging for prechronic and chronic toxicity and carcinogenicity studies (7). Their longer life span and low rate of spontaneous tumor development, especially when on a controlled diet, make them an excellent model for these types of studies. This study consisted solely of male rats because the overwhelming majority of shrapnel injuries involve males. However, the sex of the animal is not considered significant for nickel (and likely not with any heavy metals) because it is not sex linked (8).

Tumor size. Rats were assessed for both the presence of a tumor and neoplastic areas, measured in μm^2, as shown in Table 1. No tumors developed in the Ta negative controls (n=12) at 1, 3, or 6 months. One-hundred percent of

the Ni positive controls developed large tumors (n=6, 8 x 10^7 μm^2 , SEM +/- 1 x 10^7 μm^2) by 6 months as did both the WA high dose (n=6, range 2 x10^8 μm^2, SEM +/- 2 x 10^7 μm^2) and WA low dose (n=6, range 5 x 10^7 μm^2 , SEM +/- 8.8 x 10^6 μm^2) groups at 6 months. The WA low dose group developed neoplastic areas more slowly with 66.7% (n=6, range 3.2 x 10^6 μm^2, SEM +/- 4.9 x 10^5 μm^2) having visible changes at 1 month and 83.3% (n=6, range 3.4 x 10^6 μm^2, SEM +/- 5.9 x 10^5 μm^2) exhibiting changes at 6 months. The WA high dose group had neoplastic areas in 100% (n=6, range 8.9 x 10^6 μm^2 , SEM +/- 1.9 x 10^6 μm^2) of the 1 month group and 60% (n=5, range 2 x 10^7 μm^2, SEM +/- 4 x 10^7 μm^2) of the 3 month group. Neoplastic changes developed more rapidly in the WA high dose group and grew faster than those in the WA low dose group.

The neoplastic regions in the WA animals showed areas of increased invasion of muscle tissue by the neoplastic cells often separating the individual fibers. This occurred to a lesser extent with Ni.

Mitotic figures. Because there is a useful correlation between the number of mitotic figures and the biological behavior of a tumor (9), the number of mitotic figures was counted in 10 consecutive high power fields (HPF) (Figure 2). Figures counted were near the outermost edge of the tumor where it joins the muscle fibers because this area is considered to be the most proliferative (28). The mitotic rate was determined using H&E staining (10). Mitotic figures were counted near the pellet site in rats that did not have tumors. Ta (n=12; mean 0 SEM +/-0) had no mitotic figures, whereas Ni (n=6, 21.67 +/- 11.67) was significantly greater. WA low dose had more mitotic figures (13, 18.5, and 21.83)

at 1, 3, and 6 months than did WA high dose (2.5, 1.6, and 8.5) (Table 1) and even more than Ni.

Necrosis. The incidence of tumor necrosis was determined through histological examination of cross sections of the tumor. Generally, tumors with greater than 20% necrotic area are considered positive for tumor necrosis (16). The controls included 0% of Ta and 83% of Ni subjects positive for necrosis. Overall, 50% of WA low dose and 35% of WA high dose were positive for necrosis with both having increasing percentages of animals positive for necrosis at each time point (Table 1).

Apoptosis. The involvement of apoptosis in muscle damage and tumor development after implantation of Ta, Ni, and WA was examined by TUNEL staining. In the Ta negative controls, no apoptotic cells were observed. In comparison, the number of apoptotic cells was markedly increased in the Ni positive control group. In the WA high and low groups there were marked increases at 1 and 6 months (2% and 3%, respectively) (Figure 3). This was confirmed by microscopic examination of the tissue. The examination revealed the presence of convoluted and shrunken cells as well as apoptotic bodies (Figure 1).

Vascularity. Vessels were counted as the number of capillaries per fiber. The mean vessel count in the muscle surrounding the Ta group started at 0.2 per fiber, increased to 0.34 at 3 months and then dropped to 0.16. This was markedly different from the WA low-dose group, which exhibited a steady increase in vessel per fiber from a mean of 0.16 to 0.31 to 0.47 by 6 months.

The WA high-dose group actually had the population of capillaries at 0.35 per fiber (more than the Ta at any time point), stayed the same at 0 and 3 months and decreased to 0.27 at 6 months (Figure 4).

Disintegration of muscle fibers. Myofibers were counted as disintegrated if one or more of the follow was identified: internal nuclei, fiber vaculation, cell swelling, necrosis, and increased circularity (11). WA low- and high-dose and all Ni showed signs of disintegration while Ta did not. As early as 1 month, 11% of the WA low-dose fibers showed signs of disintegration. WA high dose had a greater percentage of disintegrating fibers at all time points than WA low dose (Table 1); and by 6 months 78% of all WA high dose fibers showed signs of disintegration.

Discussion

It is well established that rhabdomyosarcoma can be successfully studied in rats and that the cell variety produced resembles what is observed in humans, thus indicating that the animal rhabdomyosarcoma tumors are similar to human RMS tumors (12). In this study, nickel-implanted animals all developed rhabdomyosarcoma, similar to what has been reported by others. As is frequently the case, the most rapid area of growth is in the cells at the periphery of the tumor (12). Nickel induces rhabdomyosarcomas that are generally categorized as the pleomorphic type, which has spindled cells with strap cells exhibiting cross striations resembling myotubes (29-32). The consistency of the tumors produced by nickel exposure is why it is the ideal positive control.

There are a plethora of articles, dating from the 1950's, that demonstrate

the carcinogenicity of Ni in rats (42, 43). Sunderman et al. (13) injected F344 rats intramuscularly with 10 mg of nickel subsulfide and found sarcomas in 22 of 23 rats. They also injected hamsters in the same manner which resulted in a 59% incidence of sarcoma from intramuscular injections and 3 of 19 hamsters, by 18 months, developed rhabdomyosarcoma of the testes – an area that does not contain striated muscle. Sunderman & Meanza (14) also examined the carcinogenicity of various nickel compounds, including nickel monosulfide, nickel subsulfide, nickel iron sulfide, and nickel powder by giving each in 2 doses to determine if the compounds were equivalent. Treatment with amorphous nickel monosulfide resulted in no sarcomas while nickel subsulfide produced in tumors in most of the rats. As a result of the extensive studies using Ni compounds soluble metallic Ni is frequently used as a positive control in carcinogenicity studies (2). The use of Ni as a positive control in this study allows comparison, not only to previously published work, but also a indication of WA-induced carcinogenicity. It is also important to note that only one type of WA was investigated. There are many different tungsten-based munitions in use today and they may not all have the same carcinogenic potential.

Morphometric mapping of skeletal muscle vasculazation in physiological situations involving embedded heavy metal fragments provides a foundation for further investigations. In general, capillaries are stable structures that only have new formation when there are damaged cells or there is a need to repopulate denuded areas (15). There are 2 ways to increase capillary area in a tissue: 1) increase the diameter or length of the capillary, or 2) increase the number of

channels. Though both result in increased oxygenation of tissue, increasing the number of channels is a more effective mechanism of increasing the oxygenation of tissue and demonstrates why geometrical arrangement of capillaries is important (15).

In this instance the WA high-dose group already had a marked increase in the number of vessels per fiber as compared to the WA low-dose group at 1-month post implantation. While they were roughly equivalent at 3 months, the WA low-dose group had markedly more vessels per fiber than the WA high-dose group at 6 months and, in fact the number of vessels per fiber decreased in the WA high-dose group. Angiogenesis, the growth of new vessels, results from the interaction of mechanical factors, energy imbalance from hypoxia, and inflammatory processes (15). There is a significant amount of literature on angiogenesis and cardiac function demonstrating the importance of the 3 factors. Because red blood cells flow through the capillaries in single file separated by plasma any decrease in the number of red blood cells can result in a substantial decrease in the oxygenation of tissue and thus results in the inability to maintain a constant oxygen flux (34, 35) and in reduced conductance out of the capillary in skeletal muscle (16).

While increased capillary supply provides oxygen and nutrition needed by the tumor to grow, it is well documented that animals are only able to support a certain amount of viable tumor (17). Once that level is reached necrosis within the tumor can decrease the weight of the tumor. Interestingly, the WA high-dose group had larger tumors at all time points, but fewer were positive for necrosis

than in the WA low dose group with smaller tumors. Costa (18) categorized necrosis into 4 groups based on the percentage of the tumor with necrosis: absent, minimal did not exceed 15%, moderate was 16-50%, and massive exceeded 50%. Either by Costa's criteria or categorizing necrosis as "positive" at greater than 20%, the WA low-dose group had higher rates of necrosis at 6 months than did the WA high-dose group with the larger tumors.

Mitotic rate and extent of necrosis are prognostic factors in rhabdomyosarcoma (19). Further, they are the 2 most important parameters in grading soft tissue sarcomas (18-23). Tumors are considered to have low mitotic activity when they have fewer than 6 mitotic features per 10 high power fields (1256 μm^2) and high mitotic activity when there are 6 or more (18). The WA low-dose group had high mitotic activity at each point and more mitotic figures at each time point than the WA high-dose group. The WA high-dose group only had a high mitotic activity at 6 months. Interestingly, the WA low-dose group had higher mitotic activity than the Ni group, which is used as a positive control. Figure 2 shows clear mitotic figures in the Ni, WA high-dose, and WA low-dose groups. While cell division is required for tissue maintenance, abnormal mitoses are a sign of genetic damage. At this time it is speculative whether genetic damage or toxicity has the greatest effect in the WA high-dose group.

Cellular death occurs as the result of apoptosis, necrosis, or when cells are no longer able to maintain homeostasis due to membrane damage. Figure 3 shows a marked increase in apoptosis in both the WA high- and low-dose groups as well as apoptotic bodies and cell destruction. It is possible that inadequate

disposal of the apoptotic bodies results in inflammation, leading to destruction of surrounding cells and tumor formation.

Despite a receiving a lower dose, the WA low-dose group developed a tumor that has more mitotic figures, greater vascularity, and more apoptotic bodies than those in the WA high-dose group. It is possible that the higher overall localized concentration of Ni and Co in the WA high-dose group had a toxic effect on the surrounding tissue. This would explain the early capillary development followed by a decrease in capillaries and less necrosis. These locally high metal concentrations may cause immediate cell death in the WA high-dose group, whereas in the tungsten low-dose group lower metal concentrations and therefore less localized toxicity resulted in higher prognostic factors for rhabdomyosarcoma and greater genotoxicity. This would be consistent with the findings of Lehnert (24, 25) and explain why there is greater apoptosis and more mitotic figures with the WA low dose (the “bystander effect”).

Environmentally, when tungsten is mixed with soil at rates greater than 1%, it results in microbial changes. There is a loss of bacterial components, fungal biomass is increased and red worms die (26). Currently, the U.S. does not regulate tungsten. However, the Russian Federation does regulate with a limit of 0.05mg l^{-1} in drinking water and 0.0008 mg l^{-1} in lakes (26). Recently, there has been more interest in the U.S. after an investigation of a leukemia cluster in children in Fallon, Nevada by the Centers for Disease Control and Prevention (CDC) and various state agencies (27). While the report did not show that tungsten was the causative agent, it was found that tungsten levels in the urine of

residents were 15 times the national average. Two additional childhood leukemia clusters were found in Sierra Vista, AZ and Elk Grove, CA, both of which have tungsten mines (28).

This study, as with those previously published, raises concern about the potential health effects, including carcinogenicity, of the tungsten alloys. Based on recently published data, the greatest concern may be with embedded fragments of WA suffered as the result of a shrapnel wound. Standard surgical guidelines recommend leaving fragments in place. This study shows that even at low doses this may not be a wise decision with embedded WA. This study, while not definitive, suggests that regular monitoring of those with shrapnel injuries containing heavy metals may be warranted.

References

1. Davitt RP. A comparison of the advantages and disadvantages of depleted uranium and tungsten alloy as penetrator materials. In: Defense Do, editor.: US Army Armament Research and Development Command; 1980.
2. Refractory metals: tungsten and tungsten alloys. [cited 2007 February 2, 2007]; Available from: http://www.key-to-metals.com/Article110.htm
3. Agency for Toxic Substances and Disease Registry. Draft toxicological profile for tungsten. In: Prevention CfDCa, editor.; 2003.
4. Sciences IoLARCoL. Guide for the care and use of laboratory animals. 1996 [cited 2007 January 18, 2007]; Available from: http://www.nap.edu/readingroom/books/labratss/
5. Castro CA, Benson KA, Bogo V*, et al.* Establishment of an animal model to evaluate the biological effects of intramuscularly embedded depleted uranium fragments. Bethesda: Armed Forces Radiobiology Research Institute; 1996. Report No.: AFRRI Technical Report TR96-3.
6. Johansson CB, Hansson HA, Albrektsson T. Qualitative interfacial study between bone and tantalum, niobium or commercially pure titanium. Biomaterials 1990;11(4):277-80.
7. Chhabra RS, Huff JE, Schwetz BS, Selkirk J. An overview of prechronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. Environmental health perspectives 1990;86:313-21.
8. Friedman I, Bird E. Electron-microscope investigation of experimental rhabdomyosarcoma. Path 1967;97:375-82.
9. Cross SS, Start RD, Smith JH. Does delay in fixation affect the number of mitotic figures in processed tissue? Journal of clinical pathology 1990;43(7):597-9.
10. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. . New York: McGraw-Hill; 1968.
11. Scelsi R, Scelsi L, Poggi P. Microcirculatory changes and disuse are cause of damage to muscle fibres during aging. Basic Appl Myol 2002;12(5):193-9.
12. Maurer HM, Ruymann FB, Pochedly C. Rhabdomyosarcoma and related tumors in children and adolescents. Boston: CRC Press; 1991.
13. Sunderman FW, Jr., Maenza RM, Alpass PR, Mitchell JM, Damjanov I, Goldblatt PJ. Carcinogenicity of nickel subsulfide in Fischer rats and Syrian hamsters after administration by various routes. Advances in experimental medicine and biology 1977;91:57-67.
14. Sunderman FW, Jr., Maenza RM. Comparisons of carcinogenicities of nickel compounds in rats. Research communications in chemical pathology and pharmacology 1976;14(2):319-30.
15. Rakusan K. Coronary angiogenesis. From morphometry to molecular biology and back. Annals of the New York Academy of Sciences 1995;752:257-66.
16. Federspiel WJ, Popel AS. A theoretical analysis of the effect of the particulate nature of blood on oxygen release in capillaries. Microvascular research 1986;32(2):164-89.
17. Schatten WE. An experimental study of necrosis in tumors. Cancer Res 1962;22:286-90.
18. Costa J, Wesley RA, Glatstein E, Rosenberg SA. The grading of soft tissue sarcomas. Results of a clinicohistopathologic correlation in a series of 163 cases. Cancer 1984;53(3):530-41.
19. Ramanathan RC, A'Hern R, Fisher C, Thomas JM. Modified staging system for extremity soft tissue sarcomas. Ann Surg Oncol 1999;6(1):57-69.
20. Coindre JM, Trojani M, Contesso G*, et al.* Reproducibility of a histopathologic grading system for adult soft tissue sarcoma. Cancer 1986;58(2):306-9.

21. Myhre-Jensen O, Kaae S, Madsen EH, Sneppen O. Histopathological grading in soft-tissue tumours. Relation to survival in 261 surgically treated patients. Acta pathologica, microbiologica, et immunologica Scandinavica 1983;91(2):145-50.
22. Tomita Y, Aozasa K, Myoui A*, et al.* Histologic grading in soft-tissue sarcomas. An analysis of 194 cases including AgNOR count and mast-cell count. International journal of cancer 1993;54(2):194-9.
23. van Unnik JA, Coindre JM, Contesso C*, et al.* Grading of soft tissue sarcomas: experience of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 1993;29A(15):2089-93.
24. Goldberg Z, Lehnert BE. Radiation-induced effects in unirradiated cells: a review and implications in cancer. International journal of oncology 2002;21(2):337-49.
25. Lehnert BE, Iyer R. Exposure to low-level chemicals and ionizing radiation: reactive oxygen species and cellular pathways. Human & experimental toxicology 2002;21(2):65-9.
26. Strigul N, Koutsospyros A, Arienti P, Christodoulatos C, Dermatas D, Braida W. Effects of tungsten on environmental systems. Chemosphere 2005;61(2):248-58.
27. CDC. Cross-sectional exposure assessment of environmental contaminants in Churchill County, Nevada. Final Report. Atlanta, GA; 2003.
28. Koutsospyros A, Braida W, Christodoulatos C, Dermatas D, Strigul N. A review of tungsten: from environmental obscurity to scrutiny. Journal of hazardous materials 2006;136(1):1-19.

Tables

Table 1. Selected histological parameters for rats implanted with metal pellets at 1, 3, and 6 months.

	Months after Surgery	Ta		WA (low)		WA (high)	
Tumor Size (μm^2)	1	0	± 0	902000.0	± 492323.7	4301829	± 1915685
	3	0	± 0	1200518	± 594398.0	5875920	± 4069301
	6	0	± 0	2E+007	± 8794823	5E+007	± 2E+007
Mitotic Figures	1	0	± 0	13.00	± 7.22	2.50	± 1.20
	3	0	± 0	18.50	± 8.28	1.60	± 0.748
	6	0	± 0	21.83	± 8.27	8.5	± 2.60
Necrosis (%)	1	0	± 0	4.17	± 4.17	4.17	± 4.17
	3	0	± 0	1.42	± 8.21	10.0	± 6.12
	6	0	± 0	4.17	± 8.33	18.33	± 7.82
Vessels per Fiber	1	0.2	± .07	0.16	± 0.02	0.35	± 0.07
	3	0.34	± .03	0.31	± 0.07	0.35	± 0.08
	6	0.16	± .04	0.47	± 0.11	0.27	± 0.05
Dissintegration of Fibers (%)	1	0	± 0	11.02	± 6.72	17.89	± 16.44
	3	0	± 0	28.61	± 5.72	69.49	± 18.04
	6	0	± 0	22.81	± 7.09	77.82	± 10.95
Weight at Euthanasia (g)	1	267.77	± 7.36	300.23	± 5.83	287.12	± 7.49
	3	392.18	± 9.38	354.90	± 11.76	365.52	± 16.72
	6	454.23	± 7.19	476.25	± 10.51	419.57	± 11.15

Abbreviations: Ta, tantalum; WA, tungsten-alloy. Data represent mean +/- SEM for 12 observations for Ta, 17 for WA high dose, and 18 for WA low dose.

Figures

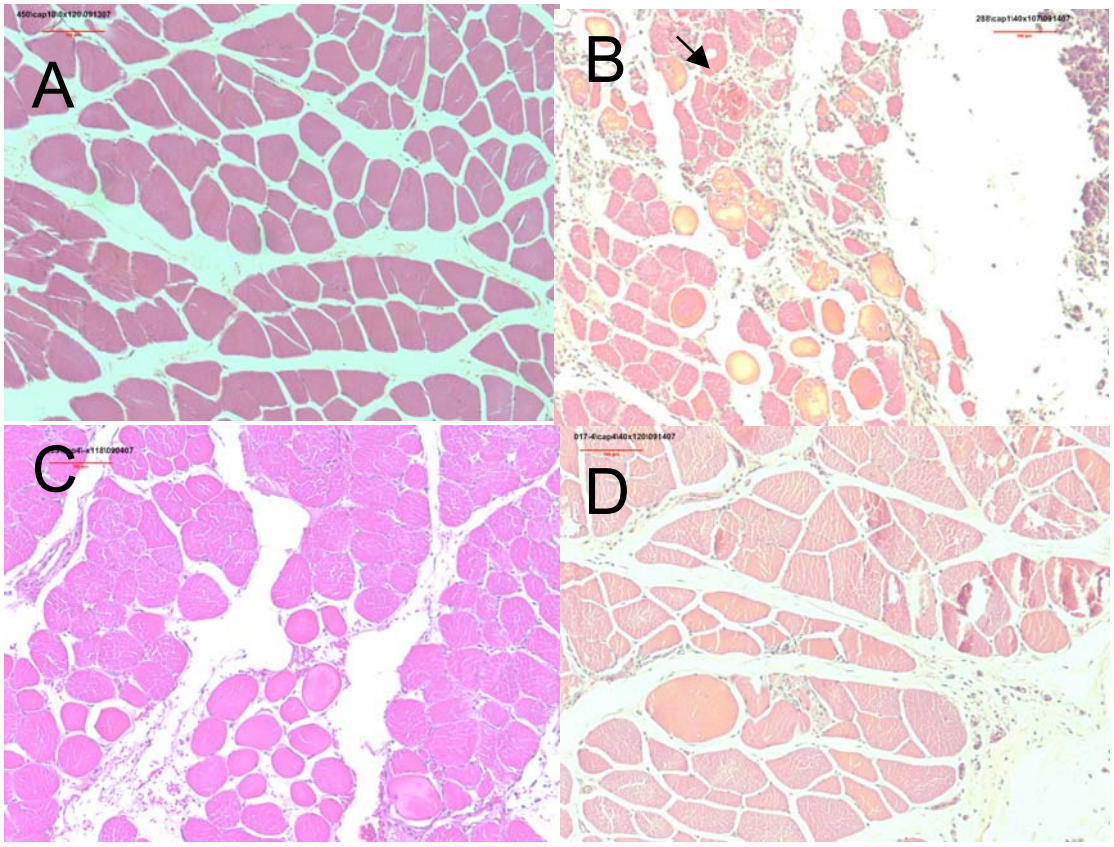


Figure 1. H&E staining of muscle tissue for rats implanted with metal pellets for 6 month. Panel **A:** Tantalum. Panel **B:** Nickel. Tissue adjacent to tumor with infiltrates between muscle fibers and classic signs of apoptosis – shrinkage and apoptotic bodies (black arrow). Panel **C:** WA-low dose. Panel **D:** WA-high dose.

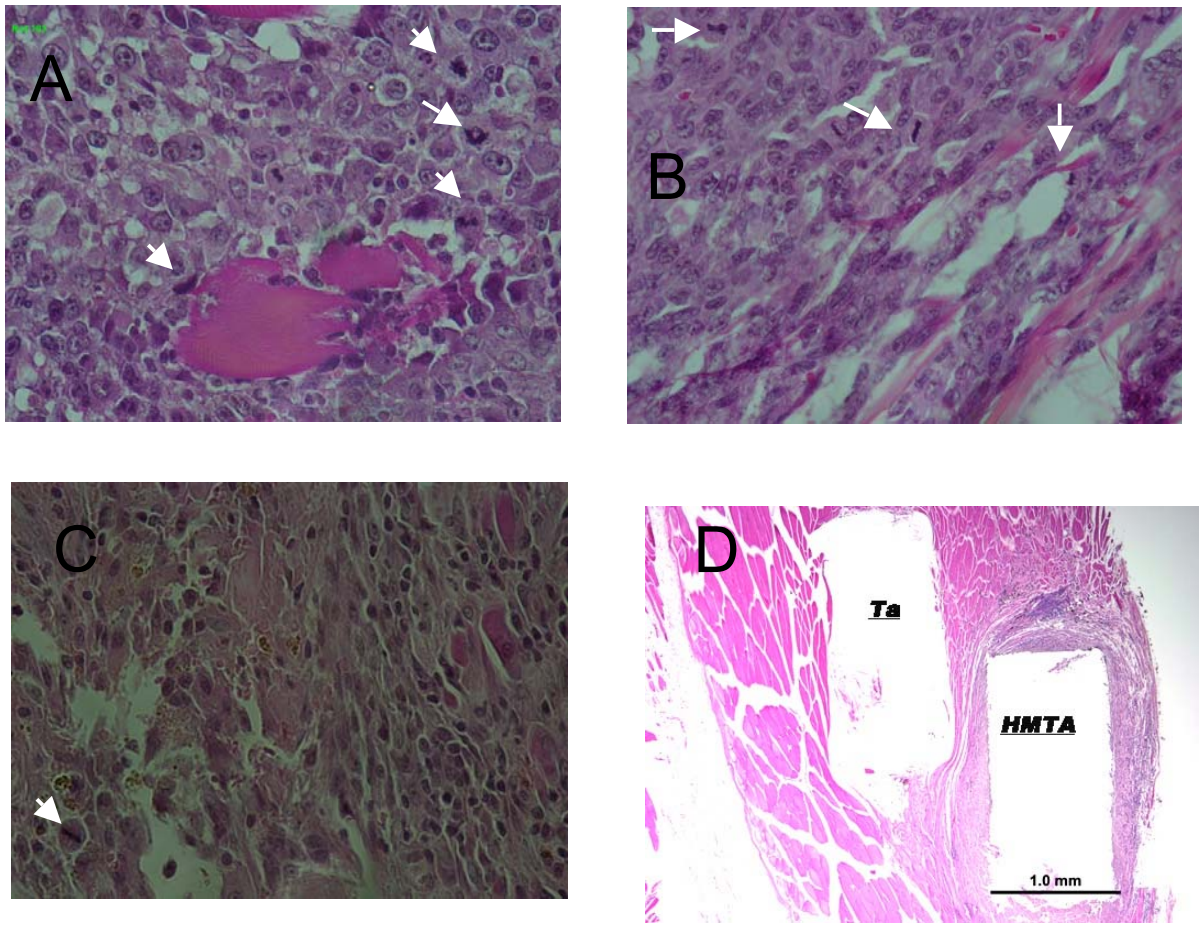


Figure 2: H&E muscle sections showing areas of high mitotic activity. White arrows indicate mitotic cells. Panel **A**: Ni group, 6 months postimplantation. Panel B: WA high-dose group, 6 months postimplantation. Panel **C:** WA low-dose group, 3 months postimplantation. Panel **D:** Tissue section from WA low-dose group showing location of a Ta pellet and a tungsten alloy (HMTA) pellet. Note the developing neoplastic area surrounding the tungsten alloy pellet.

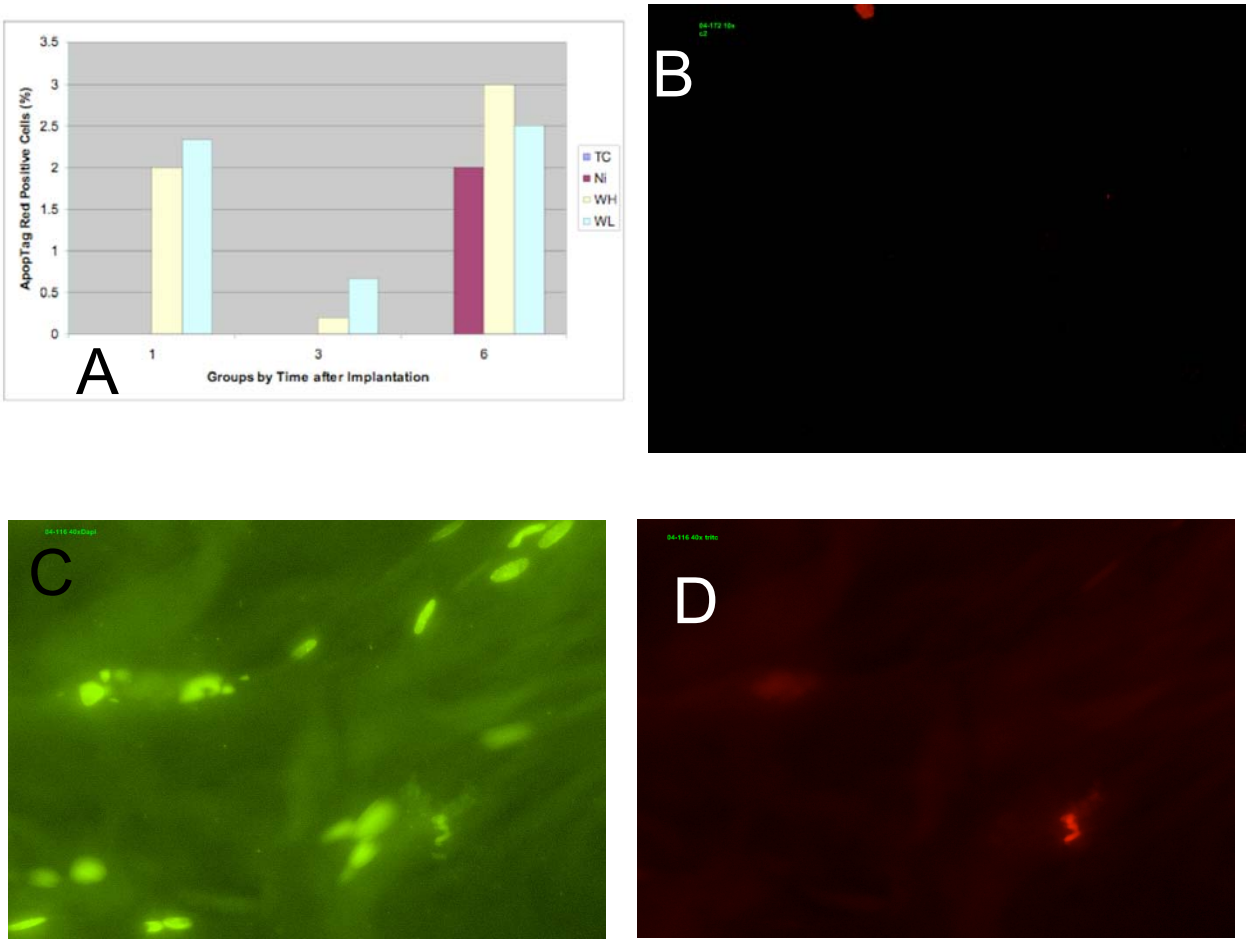


Figure 3. Effects of embedded WA on muscle apoptosis. Cell apoptosis was evaluated by described in Materials and Methods. Panel **A:** % of apoptotic cell by group and time point. Panel **B:** Muscle apoptosis in WA high-dose sample. Red spots indicate areas of DNA breakage. Panel **C:** Total nuclear staining of WA low-dose sample. **D:** Apoptotic cells in the WA low-dose sample shown in Panel C.

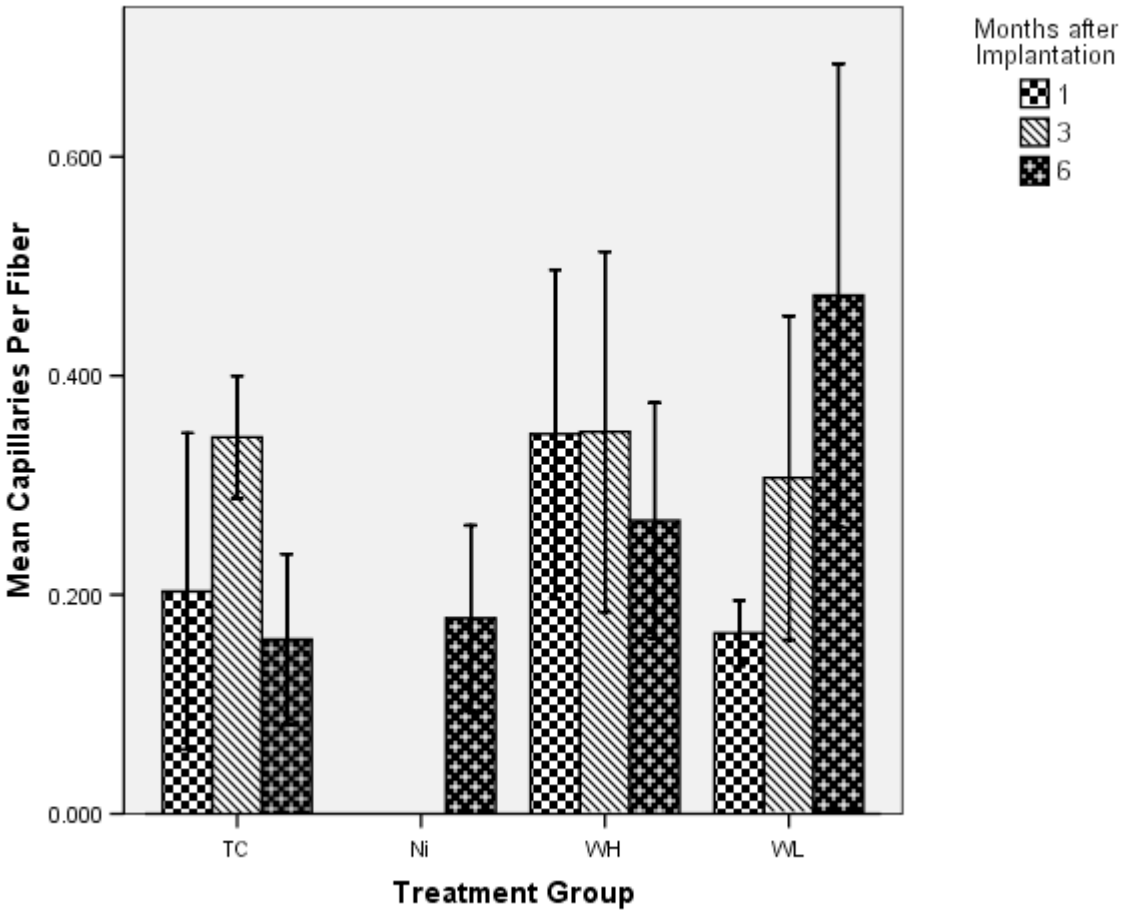


Figure 4: Effect of embedded metals on vascularity. Data are expressed as capillaries per fiber. The error bar represents SE of the mean.

Skeletal Muscle Damage as an Early Indicator of Adverse Health Effects of Tungsten Alloy

Roberta P. Lavin

Uniformed Services University of the Health Sciences

John F. Kalinich, Ph.D

Marguerite Littleton Kearney, Ph.D., RN, FAAN

Christine E. Kasper, Ph.D., RN, FAAN

No authors have any financial interest to report

Corresponding Author: Roberta P. Lavin, Graduate School of Nursing,

Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd.

Bethesda, MD 40318, rlavin10@mac.com

[Insert word count for text only]

The views expressed in this article are those of the author and do not necessarily reflect those of the U.S. Department of Health and Human Services or the Uniformed Services University of the Health Sciences. This work was supported in part by United States Army Medical Research and Materiel Command Grant DAMD17-01-1-0821. There is no conflict of interest in this publication.

Background: The use of unique metal mixtures on today's battlefields opens the possibility of embedded fragment wounds with metals whose health effects are not completely understood. For example, a tungsten alloy (WA) comprised of tungsten, nickel, and cobalt has recently been shown to induce rhabdomyosarcomas (RMS) when implanted in the legs of laboratory rats. In general, little is known about the morphologic changes that occur in skeletal muscle fibers as a result of intramuscularly embedded metal, and nothing is known about skeletal muscle changes as the result of WA. This study addresses some of those changes.

Methods: Male F344 rats were intramuscularly implanted intramuscularly with pellets (1 mm x 2 mm) of tantalum, nickel, or munitions-grade tungsten alloy containing tungsten/nickel/cobalt. Muscle samples were obtained at 1, 3, and 6 months postimplantation and assessed for a variety histologic and ultrastructural changes.

Results: No tumors developed in the tantalum-implanted (negative control) animals and no indications of neoplastic changes were seen around the implanted pellet. 100% of the Ni-implanted rats (positive control) developed large tumors by 6 months as did both groups (low dose and high dose) of tungsten alloy-implanted rats. Tumor development

was slower in the tungsten alloy low-dose group. Implantation with tungsten alloy pellets also resulted in increased vascularity, high mitotic cell number, increased collagen formation, and atrophy of the adjacent muscle.

Conclusions: There are significant histologic changes in skeletal muscle which occur prior to tumor growth and following exposure to implanted shrapnel. The results of this study while not definitive, suggests that there are significant changes in skeletal muscle structure prior to the development of rhabdomyosarcoma suggesting that regular monitoring of individuals with embedded heavy metal shrapnel is warranted.

Keywords: Cancer, tungsten alloy, heavy metal, muscle atrophy

Introduction

Skeletal muscle is well known to change its structure and function as a result of extended bed rest, immobilization, and denervation (Hikida, 1988; Reiser *et al.*, 1988; Savolainen *et al.*, 1988; Schmalbruch *et al.*, 1991). However, few studies have examined the effects of embedded metals, such as those from shrapnel injuries, on skeletal muscle. Kalinich and associates (2005) investigated the potential health effects of tungsten alloy (WA) using a rodent model developed to mimic depleted uranium (DU) shrapnel wounds in soldiers from the First Persian Gulf War. The dramatic findings suggested a strong link between WA and pleomorphic rhabdomyoscarmas (RMS) that metastasized to the lungs (Kalinich *et al.*, 2005); however, the morphologic changes, which precede the development of tumors have not been examined.

The findings of Kalinich and associates (2005) raise serious concerns about the health effects of tungsten/nickel/cobalt alloys in munitions. Shrapnel is generally left *in* situ unless it is either a large fragment or located where it is likely to cause further damage if not removed (Peyser *et al.*, 2006). While there is literature, mostly in the form of case studies, that addresses long-term health effects, none specifically addresses ultrastructure and morphological alterations in skeletal muscle following the embedding of heavy metals and prior to the appearance of RMS.

In this study we compared the effects of WA shrapnel injuries on skeletal muscle to the biologically inert metal, tantalum (Ta) and the carcinogenic metal, nickel (Ni) using a Fisher 344 (F344) rodent model developed by the Armed

Forces Radiobiology Research Institute (AFRRI). The aim of this study was to identify early changes in skeletal muscle in rats implanted with WA to determine if early morphologic changes of skeletal muscle occur prior to tumor development. Awareness and early identification of the changes may provide healthcare professionals with additional knowledge necessary to monitor for potential adverse health effects of embedded WA.

Methods

This research was conducted under an Institutional Animal Care and Use Committee (IACUC) approved protocol and all rats were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (Kalinich *et al.*, 2005). The methods for this study have been previously published by Kalinich et al. (2005) and are therefore only briefly described in this section.

Fischer 344 male rats (Harlan, Frederick, MD, USA, 6 weeks of age) were used for implantation. Upon receipt, all rats were screened for common rodent pathogens and were randomly assigned to groups. A negative control group (n=12) was implanted with tantalum (Ta) pellets, a positive control group (n=6) was implanted with nickel (Ni) pellets, and two experimental groups were implanted with either a high dose (20 pellets, n=17) or low dose (4 pellets, n=18) of WA pellets. The number and dimensions of the pellets (WA, Ni, and Ta) was based on research previously conducted at AFRRI (Castro *et al.*, 1996). Each rat received a total of 20 pellets. Rats in the low-dose WA group were implanted

with 16 Ta pellets along with the 4 WA pellets; thus metal loads across all groups were identical. The pellets were cylinders 1 mm in diameter x 2 mm in length. WA pellets consisting of 91.1% tungsten, 6% nickel, and 2.9% cobalt, similar to the tungsten alloy used in kinetic-energy penetrators, were purchased from Aerojet Ordnance Tennessee (Jonesborough, TN, USA). Tantalum (99.95% Ta) and nickel pellets (99.99 % Ni) were purchased from Alfa Aesar (Ward Hill, MA, USA). Tantalum was chosen as the implantation control metal because it is biologically inert (Johansson *et al.*, 1990) with a mass similar to tungsten, and it is used frequently in human prostheses (Hockley *et al.*, 1990; Maurer *et al.*, 1991). Rats were euthanized at 1, 3 and 6 months and muscle and tumor samples collected.

Rats were fed a certified NTP-2000 diet (Quality Lab Products, Elkridge, MD, USA) to prevent excessive weight gain and to enhance longevity (Rao, 1996). Acidified water (pH 2.5-2.8, using HCl) was provided ad libitum. Rats were paired housed in microisolator cages and were on a 12-hour light/dark cycle, with no twilight.

Surgery. Rats were implanted at approximately 9 weeks of age. Prior to surgery, all pellets were cleaned and chemically sterilized. Pellets were implanted in each gastrocnemius muscle spaced approximately 1.5 mm apart on the lateral side of each leg. Rats were closely monitored following surgery until they were ambulatory. An analgesic (buprenorphine hydrochloride, Reckitt and Colman, Hull, UK) was administered preoperatively and then as needed postoperatively. Surgical sites were examined for signs of inflammation,

infection, and local metal-induced toxicity daily for 2 weeks following surgery and weekly thereafter for the duration of the study.

Histopathology. Tissue samples from the gastrocnemius muscle were taken for histopathology examination. Gastrocnemius muscle and tumors were fixed in buffered formalin, processed and embedded in paraffin and sectioned on a microtome at 5-6 μm, mounted, and stained with hematoxylin and eosin (H&E) or gomori trichrome (Vassilopoulos *et al.*, 1977; Manta *et al.*, 1987). Disintegration of muscle fiber, change in vasularity, fiber size, amount of connective tissue, circularity, area of neoplastic tissue, number of mitotic figures, and areas of necrosis were determined by microscopic examination of the tissue.

Results

Muscle samples were studied from 53 rats that had been surgically implanted with metals (WA, Ta, or Ni) in gastrocnemius muscle. Gastrocnemius muscle was taken from all rats at each time point with the exception of the Ni-implanted group, which only had samples at 6 months post-implantation. The Fischer 344 rat was used for this study as they are frequently used in cancer research, toxicology studies, the National Toxicology Program's Carcinogen Bioassay Program, and the National Institute on Aging for pre-chronic and chronic toxicity and carcinogenicity studies (Chhabra *et al.*, 1990). Their longer life span and low rate of spontaneous tumor development, especially when on a controlled diet, make them an excellent model for these types of studies (Fisher & Brown, 1998). Only male rats were studied as the overwhelming majority of shrapnel injuries involve males. However, the sex of the animal is not

considered significant in association with cellular responses to nickel, and unlikely with any heavy metals (Friedman & Bird, 1967).

As previously reported (Kalinich *et al.*, 2005) 100% of rats with WA pellets developed aggressive, metastatic, pleomorphic RMS by 6 months post-embedding of metals. This study examined early morphologic changes prior to the actual development of a tumor.

Morphology for the Ta control group at one month post-embedding was without significant change; however, both the WA low and high-dose groups revealed muscle fibers of widely variable sizes and demonstrated loss of an angulated shape (Figure 1). Additionally, rats subjected to high-dose WA were found to have vacuoles in the myofiber samples.

Neoplastic changes. Rats were assessed for evidence of tumor and neoplastic tissue, measured in μm^2, as shown in Table 1 (all data reported as mean ± SEM). No tumors developed in the Ta negative controls (n=12) at 1, 3, or 6 months. All of the Ni positive controls developed large tumors (n=6; 8×10^7 $\mu m^2 \pm 1 \times 10^7$ μm^2) by 6 months as did both the WA high dose (n=6; 2×10^8 μm^2 $\pm 2 \times 10^7$ μm^2) and WA low dose (n=6; 5×10^7 $\mu m^2 \pm 8.8 \times 10^6$ μm^2) groups at 6 months. The WA low dose group developed neoplastic areas more slowly with 66.7% (n=6; 3.2×10^6 $\mu m^2 \pm 4.9 \times 10^5$ μm^2) having visible changes at 1 month and 83.3% (n=6; 3.4×10^6 $\mu m^2 \pm 5.9 \times 10^5$ μm^2) exhibiting changes at 6 months. The WA high dose group had neoplastic areas in 100% (n=6; 8.9×10^6 $\mu m^2 \pm 1.9 \times 10^6$ μm^2) of the 1-month group and 60% (n=5; 2×10^7 $\mu m^2 \pm 4 \times 10^7$ μm^2) of the 3-month group. Neoplastic changes developed more rapidly in the WA high dose

group and grew faster than those in the WA low dose group.

The neoplastic changes and tumors in the WA animals showed areas of increased invasion with muscles split by invading neoplastic cells. This occurred to a lesser extent with Ni.

Collagen. To further characterize and identify fibrotic and dystrophic changes in the skeletal muscle tissue gomori trichrome was used to stain the collagen blue-green and the muscle fibers red. The Ta control showed minimal increases in the about of collagen present from 1 to 6 months. Interestingly, both the WA high and low dose groups showed notable changes by 1-month post implantation and prior to the development of tumors (Figure 2). However, the changes were greater in the WA high-dose group. Fibrous changes occurred to a greater extent adjacent to the pellet implantation site and decreased with distance from the implantation site. These changes differ from Ni controls, which do not show this effect and with the exception of the neoplastic area show no significant difference in collagen deposition from Ta controls.

Fiber cross-sectional area. Fiber cross-sectional area was measured in fibers (n = 200-300 per section) adjacent to the site of pellet implantation and outside the area of neoplastic changes. Fiber size increased in the Ta control group from 1 month (n=3, 1575μm^2 ± 191) to 6 month (n=3; 2123μm^2 ± 445). The WA low dose (n=6; 1459μm^2 ± 181) and high-dose groups (n=6; 1747μm^2 ± 426) exhibited significant atrophy by one month in comparison to Ta controls. Three months post-implantation the findings are quite different. While Ta control has a large increase in fiber size (2667μm^2 ± 327), WA low dose (1840μm^2 ±

412) and WA high dose (1968μm^2 ± 279) had a much small increase in fiber size (see Figure 3).

Vascularity. Vessels per fiber were counted. The mean number of vessels per fiber in the muscle surrounding the Ta control group (0.2 per fiber), increased to 0.34 at 3 months, then dropping to 0.16. This was markedly different from the WA low-dose group, which exhibited a steady increase in vessels per fiber from 0.16 to 0.31 then to 0.47 by 6 months. In fact, the WA high-dose group had the highest starting point with 0.35 vessels per fiber (more than the Ta at any time point), remained the same at 3 months, but decreased to 0.27 by 6 months (Figure 4).

Disintegration of muscle fibers. Direct indicators of muscle damage included disruption of contractile tissue (Faulkner *et al.*, 1980). Myopathic changes observed in muscle histology include internal nuclei in the muscle fibers, degeneration and vacuolation, and necrosis in muscle fibers (Scelsi *et al.*, 2002; Yimlamai, 2004). Myofibers were counted as disintegrated if one or more of the follow was identified: internal nuclei, fiber vaculation, cell swelling, necrosis, and increased circularity. WA low- and high-dose and all Ni samples showed signs of disintegration while Ta did not. As early as 1 month, 11% of the WA low-dose fibers showed signs of disintegration. Compared to WA low-dose, WA high-dose had a greater percentage of disintegrating fibers at all time points than WA low dose (Table 1). By 6 months 78% of all WA high dose fibers showed signs of disintegration.

Nuclei. Nuclei were counted in 200 – 300 fibers and numbers expressed

per fiber. Nuclei that did not touch the sarcolemma, and were inside the myofiber were considered designated centrally located nuclei (Gallegly *et al.*, 2004). In WA low-dose (0.97 ± 0.15) and high-dose (0.92 ± 0.28) tissue the nuclei per fiber were initially lower than in Ta (1.3 ± 0.09). At three months, still prior to tumor development, the number of nuclei per fiber in WA low dose (1.45 ± 0.33) increased while the WA high dose (0.61 ± 0.28) had decreased substantially. However, by 6 months WA low dose (1.75 ± 0.21) and WA high dose (1.47 ± 0.24) were higher than Ta (0.93 ± 0.76) (Figure 5).

Mitotic figures. The mitotic rate was determined using H&E staining (Kasper *et al.*, 1996). Mitotic figures counted were near the outermost edge of the tumor where it joins the muscle fibers as this area is considered to be the most proliferative (Luna, 1968). Mitotic figures were counted near the pellet implantation site in rats without tumor formation. Ta (n=12; mean 0 SEM +/-0) had no mitotic figures, whereas Ni (n=6, 21.67 ± 11.67) was significantly greater. WA low dose had more mitotic figures (13, 18.5, and 21.83) at 1, 3, and 6 months as compared to WA high dose (2.5, 1.6, and 8.5) and even more than Ni.

Discussion

Traditional histochemical methods were used to identify changes in skeletal muscle occurring prior to the development of RMS in rats implanted with WA. The four major findings from this study are: 1) atrophy as early as 1-month post implantation, 2) neoplastic changes as early as 1-month post implantation, 3) a marked destruction of myofibers starting at 1-month post-implantation, and 4) an increase in collagen spreading outward from the pellet implantation site as early

as 1-month post implantation.

Muscle atrophy appeared consistently in all animals implanted with WA. It is well know that muscle atrophy occurs when there is a decrease in mobility or muscle use (Kasper *et al.*, 1990; Carpenter, 2001), as a result of the aging process (Rakusan, 1995), and in cancer cachexia (Espat *et al.*, 1994). Over time atrophy results in a loss of muscle mass due to a decrease in the number of myofibrils and fibers per muscle (Armstrong & Phelps, 1984). Not surprisingly, we found an overall marked decrease in myofiber size over Ta controls as early as 1-month post implantation. The initial small muscle fiber size of all animals may be due to the implantation process and recovery process; however, this would not explain the small size at 3 and 6 months for the WA rats. While the WA low-dose had an average fiber size of 1459 μm^2, at 1-month, the WA high-dose had a larger fiber size of 1747μm^2. This is significantly smaller than what has been reported previously in untreated rats. Armstrong and Phelps (1984) found that the superficial gastrocnemius muscle had an average size of 2,755 μm^2 for red, 2,145 μm^2 for middle, and 1,922 μm^2 for white fibers. While the pellets in our rats were initially placed in the same location of the gastrocnemius muscle they migrated and so were not all in the same region of the muscle; however, the fibers were significantly smaller than what was found by Armstrong for red and middle and in most cases white. Moreover, the size of the fibers may not fully represent what is occurring in the muscle. Microscopic examination showed that the cells were swelling, increasing in circularity, and some had internal nuclei and blebs – all signs of apoptosis and necrosis.

Loss of fiber mass is not correlated with the myonuclear number and fiber cross-sectional area in older animals (Manta et al., 1987; Vassilopoulos et al., 1977), but is correlated in younger ones (Gallegly *et al.*, 2004). As atrophy progresses nuclei are lost most likely from an apoptotic process (Gallegly *et al.*, 2004). Therefore it was interesting that while the myonuclear number initially started out lower in the WA groups than in the Ta control group by 3 months the number of nuclei per fiber was higher in WA low dose and by 6 months significantly higher in both WA groups.

At the same time fiber size was decreasing in WA high dose the neoplastic area was increasing and becoming necrotic for both WA low and high dose. The muscle fibers adjacent to the neoplastic areas had an elevated mitotic rate. Mitotic rate and extent of necrosis are the two most important prognostic factors in soft tissue sarcomas (Coindre et al., 1986; Costa, Wesley, Glatstein, & Rosenberg, 1984; Myhre-Jensen, Kaae, Madsen, & Sneppen, 1983; Ramanathan, A'Hern, Fisher, & Thomas, 1999; Tomita et al., 1993; van Unnik et al., 1993). The WA low-dose group had high mitotic activity at each point and more mitotic figures at each time point than the WA high-dose group. Interestingly, the WA low-dose group had higher mitotic activity than the Ni group, which was used as a positive control. While cell division is required for tissue maintenance, abnormal mitoses are a sign of genetic damage. It is unclear whether genetic damage or toxicity had the greatest effect in the WA high-dose group.

The number of satellite cells were not examined in this study, but it has

been estimated that 50% of all new cells resulting from satellite cell division become part of the muscle fiber (Brown & Stickland, 1993). Satellite cells generally divide and produce new muscle fibers only if there is a minor injury. Because of this they are believed to be key to the regenerative and adaptive potential of muscle fibers (Martins et al., 2006; Roth et al., 2001). Since the cells have a limited number of mitotic divisions it is concerning when necrosis is present because a depleted number of mitotic divisions results in incomplete regeneration (Carpenter, 2001; Mozdziak, Pulvermacher, & Schultz, 2001). Thus, the elevated number of mitotic figures resulting from the RMS further complicates muscle fiber regeneration.

Morphometric mapping of skeletal muscle vasculazation in physiological situations involving embedded heavy metal fragments provides a foundation for further investigations. In general, capillaries are stable structures that only have new formation when there are damaged cells or there is a need to repopulate denuded areas (Rakusan, 1995). Tissue capillary area is augmented by: 1) increases in the diameter or length of the capillary, or 2) increases in the number of channels. Though both result in improved tissue oxygenation, increasing the number of channels is a more effective mechanism to enhance tissue oxygenation and demonstrates why geometrical arrangement of capillaries is important (Rakusan, 1995). Our finding of a steady increase in the number of vessels in WA low dose has both a positive and a negative consequence. While the augmented blood supply aids in muscle tissue regeneration it also provides oxygen and nutrients to the tumor. Interestingly, the WA low- and high-dose

groups did not respond the in the same manner. While the WA high-dose group started out with a greater number of vessels per fiber by 6 months the number was lower.

Finally, the degeneration of muscle fiber, which was extensive, and the early formation of collagen that spread outward from the areas of neoplastic change as early as one month indicate early muscle damage even prior to 1-month. Collagen deposits reflect injury. While reports of collagen filament infiltration have been previously published (Johansson *et al.*, 1990), the distance of collagen formation from the metal surface that was observed in the current study was far greater than described by Johansson and collegues indicating that the damage associated with WA adversely impacted tissue at more distant sites than that incurred with other types of foreign body muscle injury.

It is possible that the higher overall localized concentration of Ni and Co in the WA high-dose group had a toxic effect on the surrounding tissue. This may partially explain the early capillary development followed by a decrease in capillaries and less necrosis. Locally high metal concentrations may cause immediate cell death in the WA high-dose group. The lower metal concentration in the WA low-dose group may result in localized toxicity reduction and in better prognostic factors for rhabdomyosarcoma and greater genotoxicity. This would be consistent with the findings of Lehnert (2002) and explain why there is a greater number of mitotic figures with the WA low dose (the “bystander effect”).

Conclusion

Shrapnel injuries not involving tungsten alloy can result in tumor formation

and other long-term health consequences. However, the potential expanded use of in munitions and the introduction of new materials, such as explosively-shaped charges, on the battlefield increase the risk of shrapnel wounds in combatants and noncombatants alike. It has been estimated that 60 – 70 % of all wounds result in musculoskeletal injuries (Peyser *et al.*, 2006). This study, in agreement with previously published reports, give clear indications concerning the potential health effects, including muscle atrophy, resulting from the tungsten alloys. Based on recently published data, the greatest concern may be with embedded fragments of WA suffered as the result of a shrapnel wound. Standard surgical guidelines recommend leaving fragments in place. Data from this study suggests that even at low doses leaving embedded WA fragments in play may not be a wise decision. Based on the results of this study, regular monitoring of individuals with shrapnel injuries containing heavy metals may be warranted.

However, there are many remaining unanswered questions for further investigation. The molecular triggers and signaling pathways that are causing the changes need to be identified to fully understand the cause of the severe atrophy and RMS that resulted from embedded tungsten-alloy.

REFERENCES

Armstrong RB & Phelps RO. (1984). Muscle fiber type composition of the rat hindlimb. *American Journal of Anatomy* **171,** 259-272.

Brown SC & Stickland NC. (1993). Satellite cell content in muscles of large and small mice. *Journal of anatomy* **183 (Pt 1),** 91-96.

Carpenter SK, G. (2001). *Pathology of Skeletal Muscle*. Oxford University Press, Oxford.

Castro CA, Benson KA, Bogo V, Daxon EG, Hogan JB, Jaccocks HM, Landauer MR, McBride SA & Shehaa CW. (1996). Establishment of an animal model to evaluate the biological effects of intramuscularly embedded depleted uranium fragments. Armed Forces Radiobiology Research Institute, Bethesda.

Chhabra RS, Huff JE, Schwetz BS & Selkirk J. (1990). An overview of prechronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. *Environmental health perspectives* **86,** 313-321.

Espat NJ, Copeland EM & Moldawer LL. (1994). Tumor necrosis factor and cachexia: a current perspective. *Surgical oncology* **3,** 255-262.

Faulkner JA, Niemeyer JH, Maxwell LC & White TP. (1980). Contractile properties of transplanted extensor digitorum longus muscle of the cat. *Journal of Applied Physiology* **238,** C120-C126.

Fisher JS & Brown M. (1998). Immobilization effects on contractile properties of aging rat skeletal muscle. *Aging (Milano)* **10,** 59-66.

Friedman I & Bird E. (1967). Electron-microscope investigation of experimental rhabdomyosarcoma. *Path* **97,** 375-382.

Gallegly JC, Turesky NA, Strotman BA, Gurley CM, Peterson CA & Dupont-Versteegden EE. (2004). Satellite cell regulation of muscle mass is altered at old age. *J Appl Physiol* **97,** 1082-1090.

Hikida Y. (1988). [Evaluation of tooth supporting structure function by using its step response]. *Nihon Hotetsu Shika Gakkai Zasshi* **32,** 293-307.

Hockley AD, Goldin JH, Wake MJ & Iqbal J. (1990). Skull repair in children. *Pediatric neurosurgery* **16,** 271-275.

Johansson CB, Hansson HA & Albrektsson T. (1990). Qualitative interfacial study between bone and tantalum, niobium or commercially pure titanium. *Biomaterials* **11,** 277-280.

Kasper CE, Maxwell LC & White TP. (1996). Alterations in skeletal muscle related to short-term impaired physical mobility. *Res Nurs Health* **19,** 133-142.

Kasper CE, White TP & Maxwell LC. (1990). Running during recovery from hindlimb suspension induces transient muscle injury. *J Appl Physiol* **68,** 533-539.

Luna LG. (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. . McGraw-Hill, New York.

Manta P, Vassilopoulos D & Spengos M. (1987). Nucleo-cytoplasmic ratio in ageing skeletal muscle. *Eur Arch Psychiatr Neurol Sci* **236,** 235-236.

Maurer HM, Ruymann FB & Pochedly C. (1991). *Rhabdomyosarcoma and related tumors in children and adolescents*. CRC Press, Boston.

Peyser A, Khoury A & Liebergall M. (2006). Shrapnel management. *The Journal of the American Academy of Orthopaedic Surgeons* **14,** S66-70.

Rakusan K. (1995). Coronary angiogenesis. From morphometry to molecular biology and back. *Annals of the New York Academy of Sciences* **752,** 257-266.

Rao GN. (1996). New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam Appl Toxicol* **32,** 102-108.

Reiser PJ, Kasper CE, Greaser ML & Moss RL. (1988). Functional significance of myosin transitions in single fibers of developing soleus muscle. *The American journal of physiology* **254,** C605-613.

Savolainen J, Myllyla V, Myllyla R, Vihko V, Vaananen K & Takala TE. (1988). Effects of denervation and immobilization on collagen synthesis in rat skeletal muscle and tendon. *The American journal of physiology* **254,** R897-902.

Scelsi R, Scelsi L & Poggi P. (2002). Microcirculatory changes and disuse are cause of damage to muscle fibres during aging. *Basic Appl Myol* **12,** 193-199.

Schmalbruch H, al-Amood WS & Lewis DM. (1991). Morphology of long-term denervated rat soleus muscle and the effect of chronic electrical stimulation. *The Journal of physiology* **441,** 233-241.

Vassilopoulos D, Lumb EM & Emery AEH. (1977). Karyometric changes in human muscle with age. *European Neurology* **16,** 31-34.

Yimlamai T. (2004). The effects of hindlimb unweighting and beta 2-agonist on the ubiquitin-proteasome pathway and insulin-like growth factor I. In *Exercise and Sports Sciences*. University of Flordia, Gainsville.

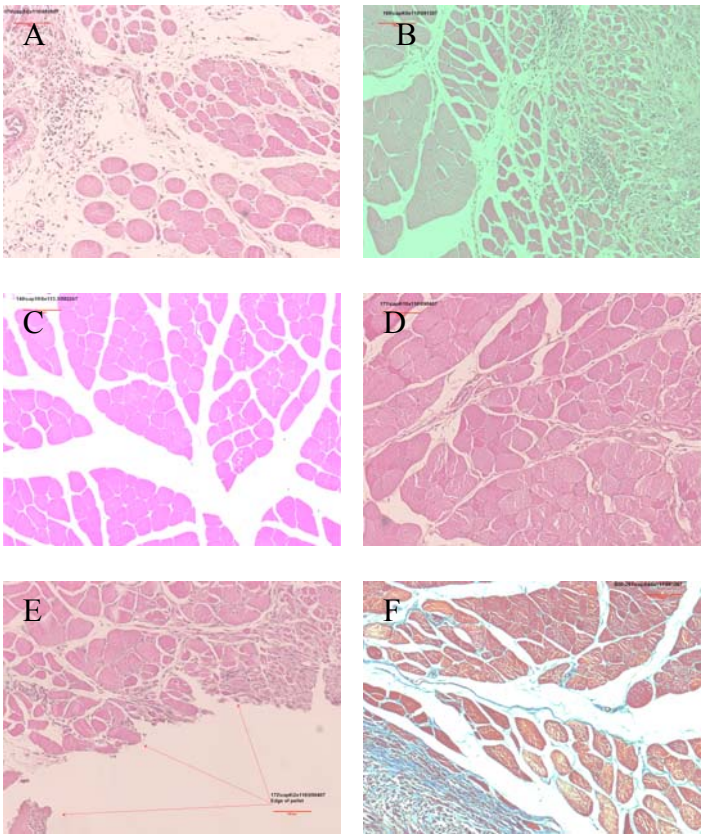
TABLES

Table 1. Selected histological parameters (mean +/- SEM) for rats implanted with metal pellets

	Months after Surgery	Ta		WA (low)		WA (high)	
Neoplastic area (μm^2)	1	0	± 0	902000	± 492323	4301829	± 1915685
	3	0	± 0	1200518	± 594398.0	5875920	± 4069301
	6	0	± 0	2E+007	± 8794823	5E+007	± 2E+007
Mitotic Figures	1	0	± 0	13.00	± 7.22	2.50	± 1.20
	3	0	± 0	18.50	± 8.28	1.60	± 0.748
	6	0	± 0	21.83	± 8.27	8.5	± 2.60
Fiber size in μm^w	1	1575	± 191	1459	± 181	1747	± 426
	3	2667	± 327	1840	± 412	1968	± 279
	6	2122	± 445	1996	± 256	1767	± 486
Vessels per Fiber	1	0.2	± .07	0.16	± 0.02	0.35	± 0.07
	3	0.34	± .03	0.31	± 0.07	0.35	± 0.08
	6	0.16	± .04	0.47	± 0.11	0.27	± 0.05
Disintegration of Fibers (%)	1	0	± 0	11.02	± 6.72	17.89	± 16.44
	3	0	± 0	28.61	± 5.72	69.49	± 18.04
	6	0	± 0	22.81	± 7.09	77.82	± 10.95
Nuclei per Fiber	1	1.3	± 0.09	0.97	± 0.15	0.93	± 0.24
	3	0.98	± 0.11	1.45	± 0.33	0.61	± 0.28
	6	0.93	± 0.76	1.75	± 0.21	1.47	± 0.28
Circularity (1 = perfect circle)	1	0.79	± 0.003	0.76	± 0.003	0.73	± 0.003
	3	0.70	± 0.003	0.74	± 0.003	0.77	± 0.003
	6	0.72	± 0.004	0.75	± 0.003	0.76	± 0.003
Weight at Euthanasia (g)	1	267.77	± 7.36	300.23	± 5.83	287.12	± 7.49
	3	392.18	± 9.38	354.90	± 11.76	365.52	± 16.72
	6	454.23	± 7.19	476.25	± 10.51	419.57	± 11.15

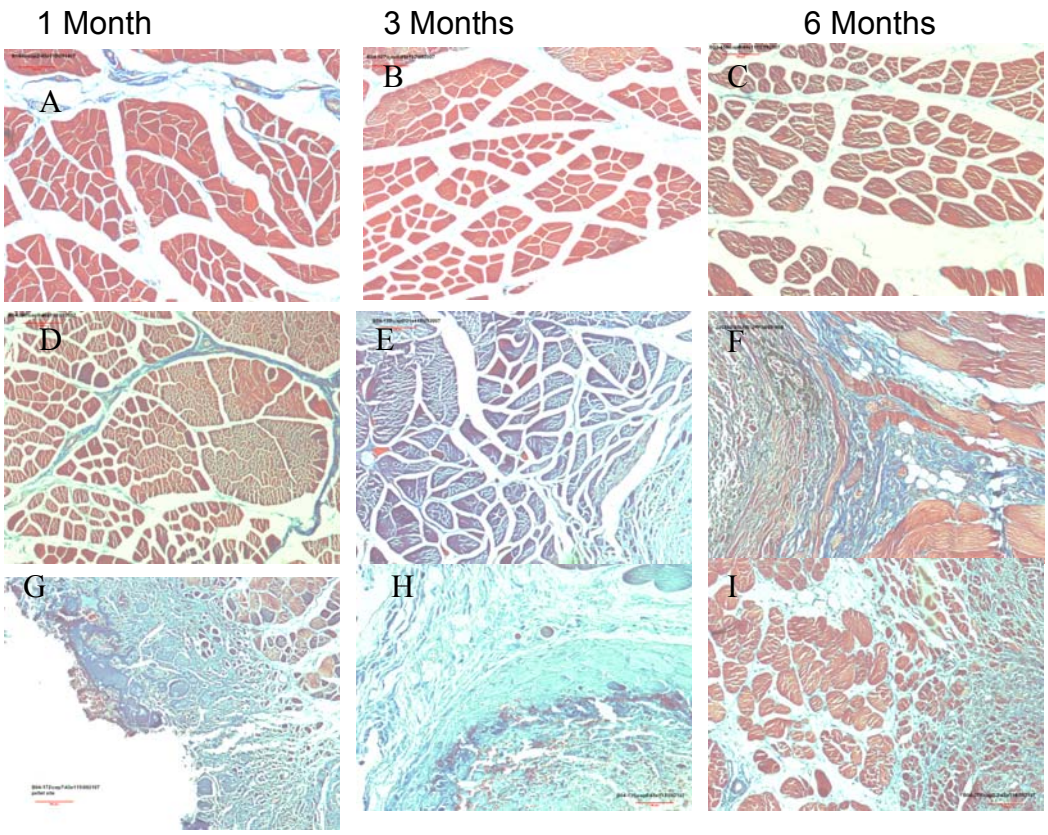
FIGURES

Figure 1. Muscle fiber changes



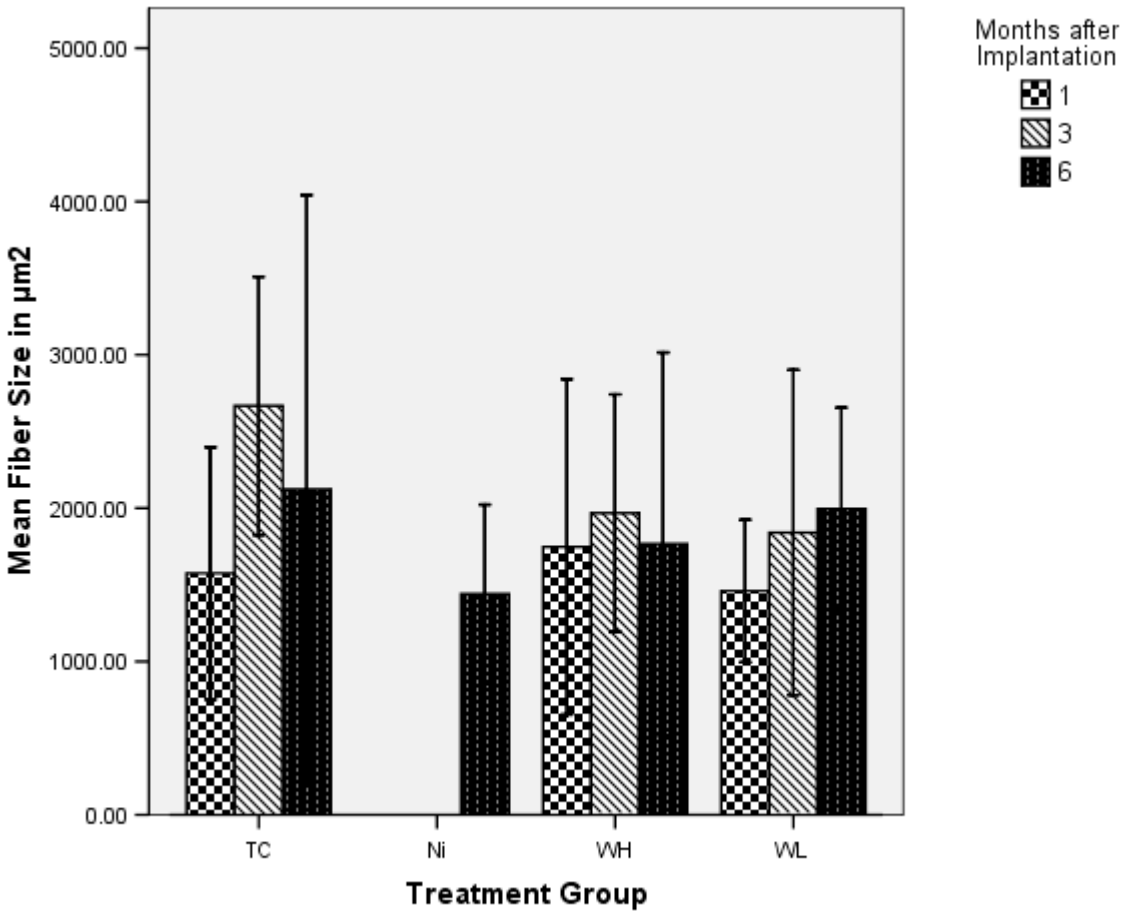
Histologic sections of gastrocnemius muscle implanted with metals. A-E (H&E, 10x) at one month post implantation. F (gomori trichrome, 10x) of Ni 6 months post implantation with increased collagen formation. A, WA high dose at 1-month shows infiltrates in spaces between the myofibers. B, WA low dose at one month shows fibers of widely varying sizes. C, Ta control apparently normal. D, WA high dose 1-month with multiple vacuoles. E, WA 1-month shows extensive destruction of fibers close to the pellet site including varying fiber size, internal nuclei, and areas of necrosis.

Figure 2. Collagen formation in rats with embedded tantalum and tungsten-alloy



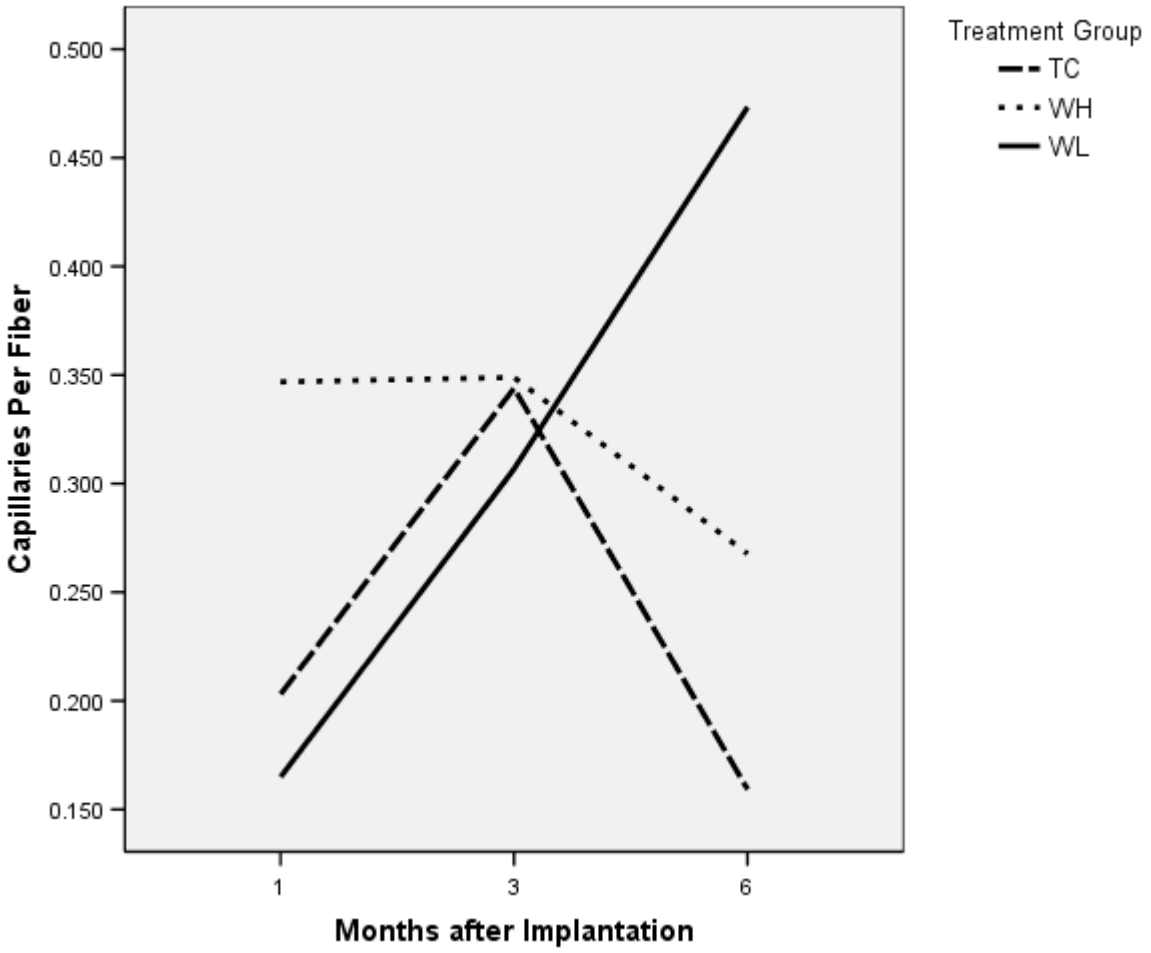
Gomori trichrome (10x) near pellet implantation site. A-C, Ta shows little collagen formation. D-F, WA low dose shows extensive collagen formation as early as 1 month. F-I, WA high dose show extensive collagen formation at 1 month.

Figure 3: Mean fiber size in μm^2 for all rats



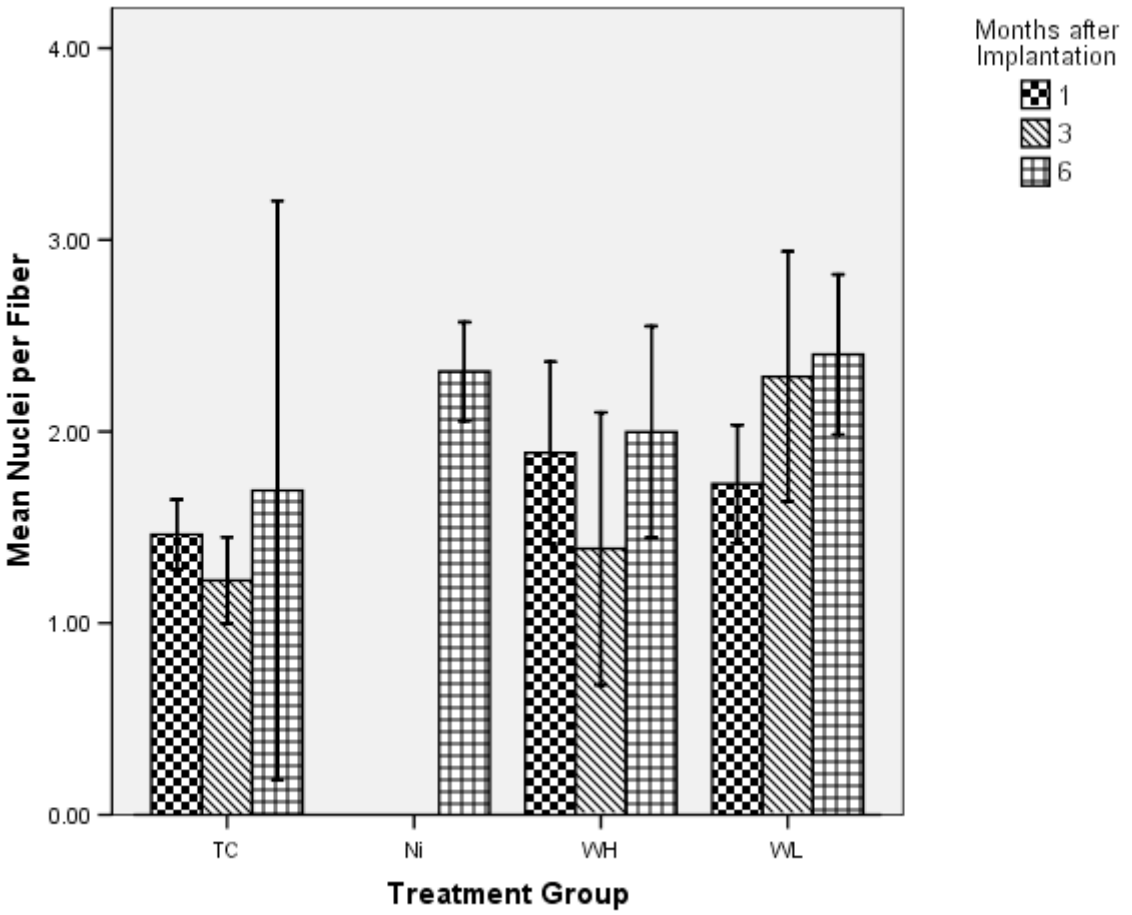
in untreated rats. Tantalum control (TC), nickel (Ni6), tungsten-alloy high dose (WH) and tungsten-alloy low dose (WL) are all represented at 1, 3, and 6 months demonstrating that all tungsten-alloy groups remain below the fiber size of the Ta control group. Error bars represent standard error of the mean.

Figure 4. Capillaries per fiber



Capillaries per fiber were counted in an area containing 200 – 300 fibers. The tantalum control (TC) group initially increased and then had a sharp decrease by 6 months. The tungsten-alloy high dose (WH) group initially stayed steady before decreasing by 6 months and the tungsten-alloy low dose (WL) showed a marked increase at 3 and 6 months.

Figure 5. Number of nuclei per fiber



Nuclei were counted in an area containing 200-300 fibers for each group. The number of nuclei per fiber was greater in the treatment groups (WL, tungsten-alloy low dose; WH, tungsten-alloy high dose) than in the tantalum control (TC). Error bars represent standard error of the mean.

Dissertation Abstract

Title: Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents
Location: Uniformed Services University
Principal Investigator: **Roberta Lavin, CAPT, USPHS**
Description: The purpose of this experiment was to identify histologic changes in skeletal muscle prior to the development of rhabdomyosarcoma (RMS) in F344 rats with embedded tungsten alloy (WA). The study explored morphologic and histologic changes that occurred prior to the development of RMS. The research objective was to provide indications of early signs of skeletal muscle changes that may indicate impending adverse consequences of embedded tungsten alloy. The primary aims of the study are: (1) to characterize the ultrastructural and morphologic forms of skeletal muscle damage characteristic of the presence of embedded WA, (2) to determine if the rate and magnitude of the development of early signs of damage or change in the ultrastructure and morphology of skeletal muscle is a precancerous indicator, and (3) to determine the rate and magnitude of the development of RMS at 3 time points. Study Population/Sample: Existing samples were obtained from a project conducted at the Armed Forces Radiobiology Research Institute (AFRRI). Study Design: Four groups of Fischer 344 male rats were used for the pellet implantation study. A negative control group was implanted in the gastrocnemius muscle with tantalum (Ta) pellets, a positive control group was implanted with nickel (Ni) pellets, and two experimental groups were implanted with either a high dose (20 pellets) or low dose (4 pellets) of WA pellets. To keep metal loads identical, those in the low-dose WA group also received 16 Ta pellets, thus every rat was implanted with 20 pellets. The number and dimensions of the pellets (cylinders, 1 mm in diameter x 2 mm in length) were based on research previously conducted at AFRRI. The WA pellets consisted of 91.1% tungsten, 6% nickel, and 2.9% cobalt, similar to one of the tungsten alloys used in kinetic-energy penetrators. Tantalum was chosen as the implantation control metal because it is biologically inert with a mass similar to tungsten. Nickel was used a positive control because it is a known carcinogen. Rats were euthanized at 1, 3 and 6 months and muscle and tumor samples collected. Findings: No tumors developed in the Ta-implanted animals and no indications of neoplastic changes were seen around the implanted pellets. 100% of the Ni-implanted rats developed large tumors by 6 months as did both the low- and high-dose-implanted rats. Tumor development was slower in the WA low-dose group. Implantation with WA pellets also resulted in increased vascularity, high mitotic cell number, and both apoptosis and necrosis of the muscle. In addition, histochemical examination of the muscle surrounding the implanted WA pellet showed severe atrophy, neoplastic changes, myofibril degradation, and collagen deposition as early as 1-month post implantation. The findings of the study will help to provide information to guide treatment decisions and potential future research.

© (1971/72); Friedensreich Hundertwasser

Doctoral Defense

Histologic Changes as Indicators of Carcinogenicity of Tungsten-Alloy in Rodents

Roberta Lavin, MSN, APRN, BC
CAPT, USPHS
Uniformed Services University of the Health Sciences
Graduate School of Nursing

Dissertation Committee

- Chair
  - Christine Kasper, PhD, RN, FAAN, FACSM
- Members
  - John Kalinich, PhD
  - Roopa Biswas, PhD
  - Marguerite Littelton Kearney, PhD, RN, FAAN

Background

- Tungsten is a naturally occurring substance
  - Blood levels of 1-6 mg/L and urine levels of 0.085 mg/L (ASTDR, 2003)
  - There is some evidence that occupational exposure to dust produced by hard metal industry may result in adverse health effects (may be cobalt and not tungsten)
  - WA is increasingly used in military munitions

Background

- Until the experiments by Miller et al. (2001) and Kalinich et al. (2005) there were few studies identifying the health effects of either oral or dermal exposure to tungsten compounds
- Kallinich et al.
  - 100% of rats implanted with WA developed pleomorphic rhabdomyosarcoma (RMS)
    - Raised serious concerns about the health effects

Weapons and Uses

- Improvised explosive devices (IED)

  More commonly used in terrorist incidents
- High velocity weapons
  - War fighting
- Low velocity weapons
  - Street violence

Wounds – Types and Locations

Table 1. Percentage of wounds by weapon type and population

	Civilian (1, 33)	Military (2-4, 15)
Low-velocity (handguns)	76 - 97	
Medium-velocity (shotguns)	3 - 12	
High-velocity (rifles)	1 - 12	28 - 55 %
Improvised Explosive Device (largely shrapnel)	0	45 - 72 %

Table 2. Percentage of wounds by location in terrorist incidents (4, 7)

	Gunshot	Explosion
Head	21	35
Trunk	39	27
Upper extremities	19	19
Lower extremities	21	19

Table 4. Complications and treatment of retained fragments

Location	Potential Problems	Recommendations
Craniocerebral	Bleeding Migration Infection	Primary debridement & wound closure for GCS 13 -15 with limited tissue destruction Primary closure without active bleeding (82) Remove shrapnel with documented migration, large objects that are accessible or pose potential risk Consider endo vascular therapy as first -line option if object crosses 2 dural compartments or involves facial or orbital regions (83)
Spinal Canal	Chronic infection Bone overgrowth Spinal cord compression (34, 84)	Remove FB > 1 cm (70) Careful histories and screening mammograms (69) Removal not recommended except in cauda equine injury (85)
Heart	Pericardium or pericardial space - pericarditis (74) Free or protruding into the cavity –endocarditis	Fragments in the myocardium, pericardium & pericardial space - leave in place Not completely embedded in the myocardium -remove Intracavitary spaces especially on the right side - monitor for movement into the pulmonary artery from where they can be removed Intracavitary or partially embedded in the mayocardium that are found late - follow to determine if they are encapsulated, if so they can be followed Large, symptomatic, or those with irregular margins especially if located next to an artery - remove (74) Asymptomatic with intracardiac fragment – manage conservatively with regular follow -up examination, but any complication require surgical intervention (16)
Pulmonary artery	Signs the fragment embolizes: pulmonary infarction, erosion or sepsis, asymptomatic (74, 86)	Some suggest observation only, others catheter extraction or thoracotomy (75, 87, 88) Removal of bullet emboli to the pulmonary artery due to risk of pulmonary complications (75) Asymptomatic – monitor (74, 86)
Abdomen	Renal colic (72)	Note GSW in history
Musculoskeletal	Joint damage; pain in weight bearing area; arthritis	Debride as much shrapnel as possible Remove fragment in joints (64) Remove devitalized tissue (89) Fragment not in joints may remain (64) Sequential x-rays
General	Damage to neurovascular structures; plubism	Remove fragment Surveillance every 3 months for 1 year Chelation therapy (33)

Multistage Model of Carcinogenicity

- Carcinogenesis is a complex and dynamic biological process that is best viewed as a system
- This classic theory of cancer is now foundational
- There are a number of cells (N) that can divide and potentially experience a carcinogenic transformation
- The kth change, which is sudden and irreversible, results in the development of cancer
- It is assumed that there is a delay between transformation to cancer and actual detection

Physiologic Rodent Model

- The rat is an excellent physiologic model for assessing the effects of embedded metal fragments
- Used as the model for metal-induced toxicological effects for many years
- Muscle is histologically similar to humans
- Extensive toxicological studies provide a good database
- Available data on HMTA is exclusively in rodents
- The size of the animal allows the rapid and technically straightforward implantation of multiple pellets without noticeable discomfort to the rat

Purpose

- The purpose of the research was to:
  - Identify early histologic changes in skeletal muscle in F344 rats that may indicate that heavy metal tungsten-alloy (WA) is causing muscle damage, and
  - Identify early changes in ultrastructure and morphology of skeletal muscle that occur prior to the development of RMS and may serve as indicators

Overall Objective

- The overall objective of this research study is to provide information about early signs of skeletal muscle changes that may indicate impending adverse consequences of embedded WA

Aims and Hypothesis

- Specific Aims
  - Characterize the ultrastructural and morphologic forms of skeletal muscle damage characteristic of the presence of embedded WA
    - The characteristics of skeletal muscle damage are significantly different in the F344 rats with embedded WA than in F344 rats with embedded Tantalum (Ta)
    - The characteristics of skeletal muscle damage are not significantly different in F344 rats with WA than in F344 rats with embedded Nickel (Ni)
      - Muscle damage was measured by disintegration of muscle fiber, loss of connective tissue, change in vascularity,change in area, and change in circularity

Aims and Hypothesis

- Determine if the rate and magnitude of the development of early signs of damage or change in the ultrastructure and morphology of skeletal muscle is a precancerous indicator
  - Skeletal muscle damage in rats with embedded WA is present prior to tumor development

Aims and Hypothesis

- Correlate the rate and magnitude of rhabdomyosarcoma development at 3 time points to the morphology and ultrastructure of the experimental muscle
  - Morphology and ultrastructure changes of the muscle with embedded WA indicative of skeletal muscle damage will significantly increase as pleomorphic rhabdomyosarcoma develops as measured by tumor size, mitotic rate, and extent of necrosis

Study Sample

- Samples were obtained from AFRRI
- Male F344 rats were randomly assigned to four experimental groups
  - Negative control (20 Ta pellets)
    - 3 (3-months), 6 (3-months), 3 (6-months)
  - Positive control (20 Ni pellets)
    - 6 (6 months only)
  - WA high dose (20 WA pellets)
    - 6 (1 & 6 months), 5 (3-months)
  - WA low dose (4 WA and 16 Ta pellets)
    - 6 (1, 3, 6 months)

Data Analysis

- Micrographs of muscle cross-sections were analyzed using ImageJ
  - Image J is a public access image process program based on NIH Image
  - Designed to allow detailed quantitative analysis
  - Widely used in a range of measurement applications
  - Has been show to be a valid and reliable alternative to manual measures (Tran, 2000)
  - All data can be statistically evaluated as scale data in SPSS

Statistical Analysis

- Muscle fiber size varies
  - Age, sex, and weight
  - All male rats are older than 81 days at the time of sampling
- Descriptive statistics were used to determine SD of all variables acquired from cross-sections
  - Data is presented as mean ± SEM

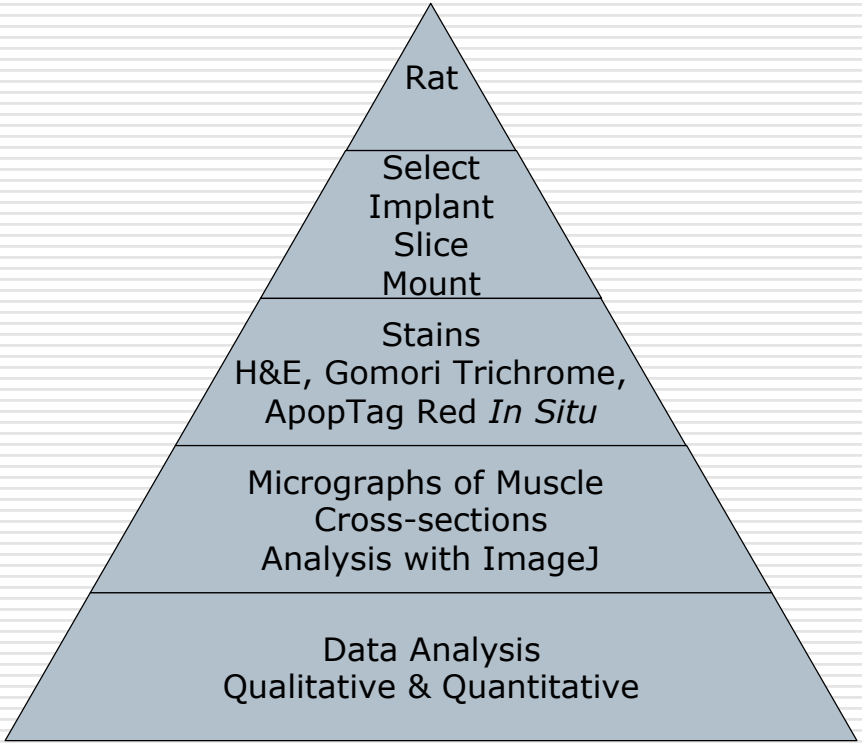
IACUC

- Maintained in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals
- IACUC approval was obtained for the original study (protocol # 0701-ACM-01.0-AID)
- Tissue does not require further IACUC approval

Federal & Military Relevance

- Military & civilian personnel increasingly face shrapnel injuries that may contain WA
- It is critical that nurses be aware of the potential indications of embedded WA
  - This knowledge provides a basis for policy changes on the removal of shrapnel, and
  - A basis to guide patient assessments – do individuals have a history of shrapnel wounds?

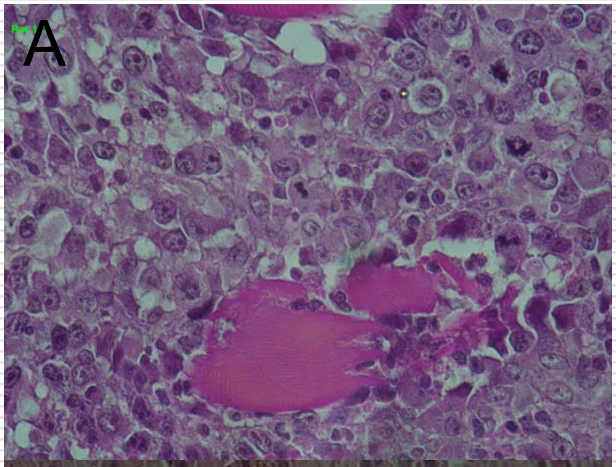
Process

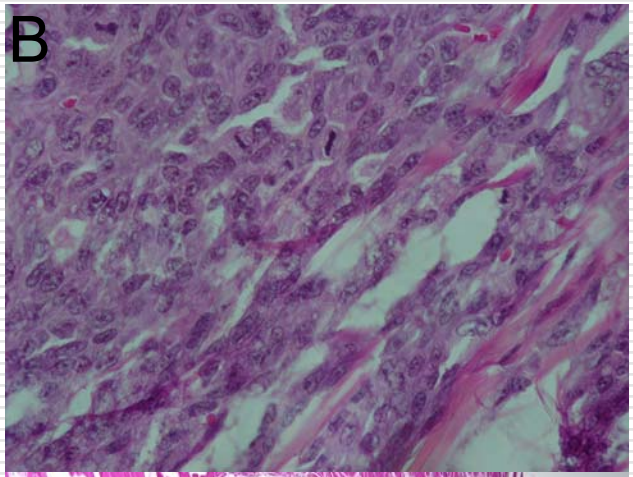

Rat
Select
Implant
Slice
Mount
Stains
H&E, Gomori Trichrome,
ApopTag Red *In Situ*
Micrographs of Muscle
Cross-sections
Analysis with ImageJ
Data Analysis
Qualitative & Quantitative

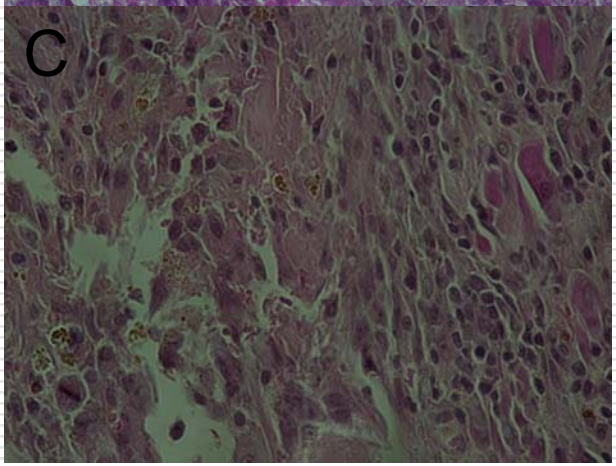
Results

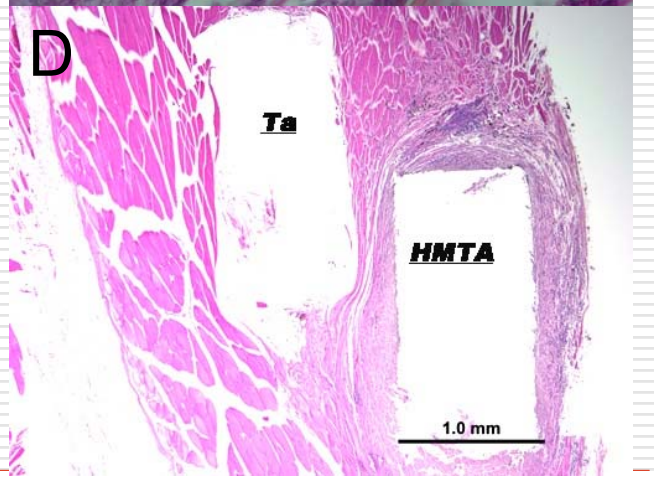
	Months after Surgery	Ta		WA (low)		WA (high)	
Tumor Size (μm^2)	1	0	± 0	902000.0	± 492323.7	4301829	± 1915685
	3	0	± 0	1200518	± 594398.0	5875920	± 4069301
	6	0	± 0	2E+007	± 8794823	5E+007	± 2E+007
Mitotic Figures	1	0	± 0	13.00	± 7.22	2.50	± 1.20
	3	0	± 0	18.50	± 8.28	1.60	± 0.748
	6	0	± 0	21.83	± 8.27	8.5	± 2.60
Necrosis (%)	1	0	± 0	4.17	± 4.17	4.17	± 4.17
	3	0	± 0	1.42	± 8.21	10.0	± 6.12
	6	0	± 0	4.17	± 8.33	18.33	± 7.82
Vessels per Fiber	1	0.2	± .07	0.16	± 0.02	0.35	± 0.07
	3	0.34	± .03	0.31	± 0.07	0.35	± 0.08
	6	0.16	± .04	0.47	± 0.11	0.27	± 0.05
Dissintegration of Fibers (%)	1	0	± 0	11.02	± 6.72	17.89	± 16.44
	3	0	± 0	28.61	± 5.72	69.49	± 18.04
	6	0	± 0	22.81	± 7.09	77.82	± 10.95
Weight at Euthanasia (g)	1	267.77	± 7.36	300.23	± 5.83	287.12	± 7.49
	3	392.18	± 9.38	354.90	± 11.76	365.52	± 16.72
	6	454.23	± 7.19	476.25	± 10.51	419.57	± 11.15

Mitotic Figures

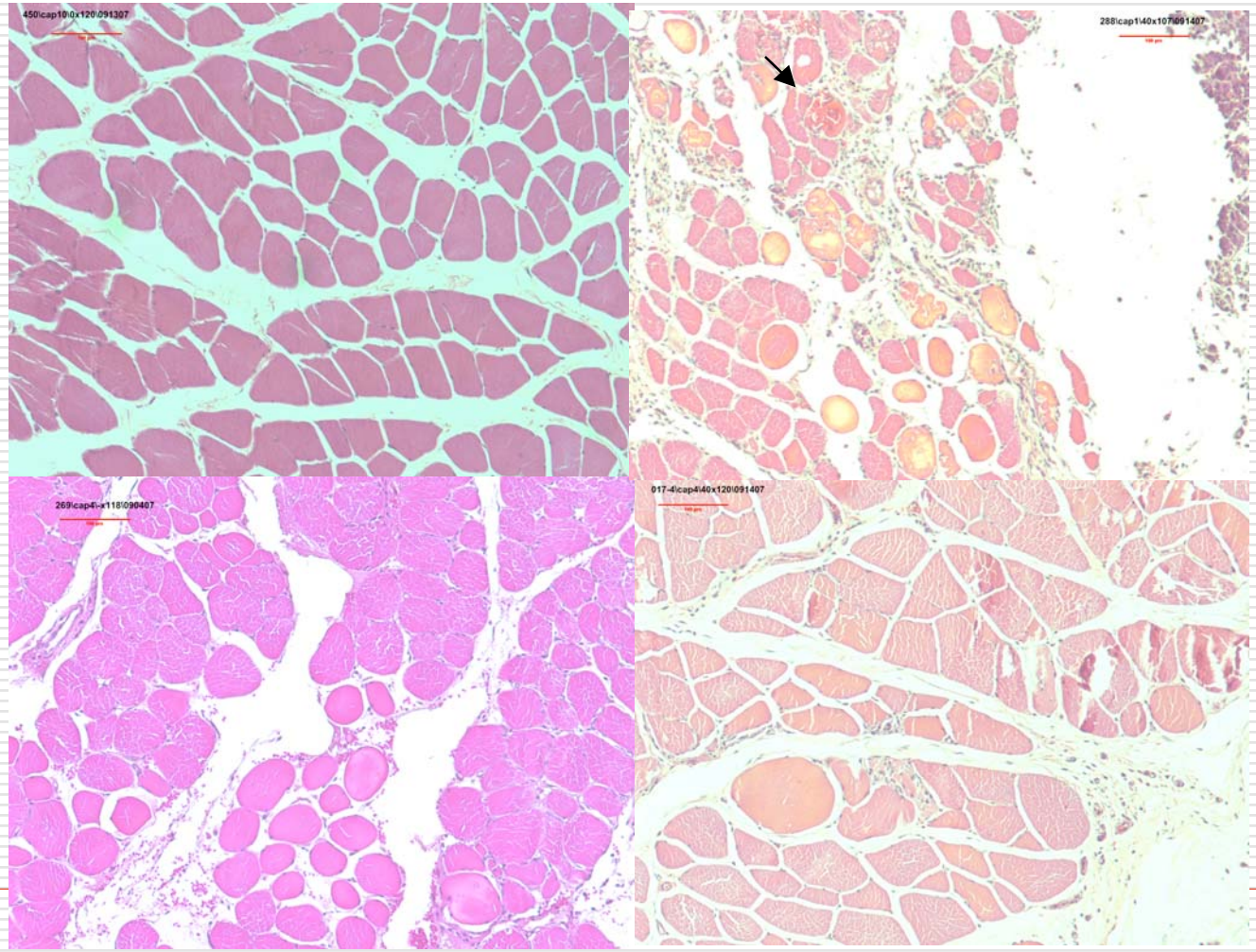




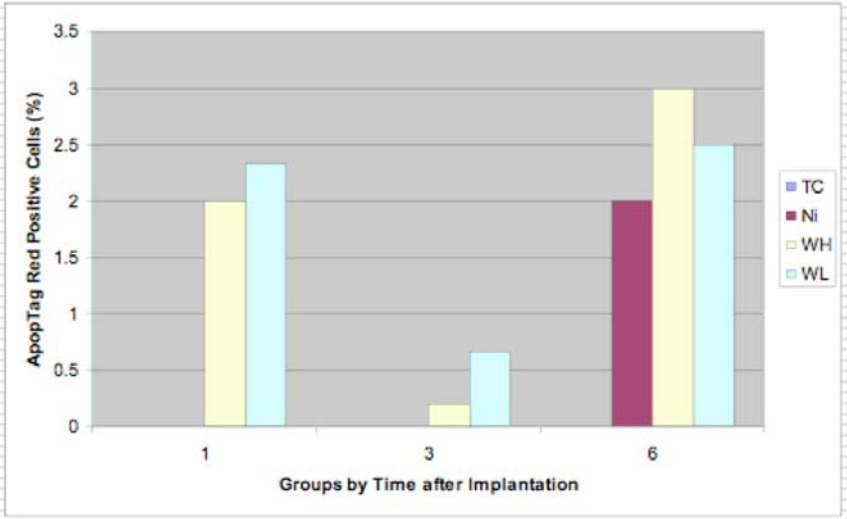


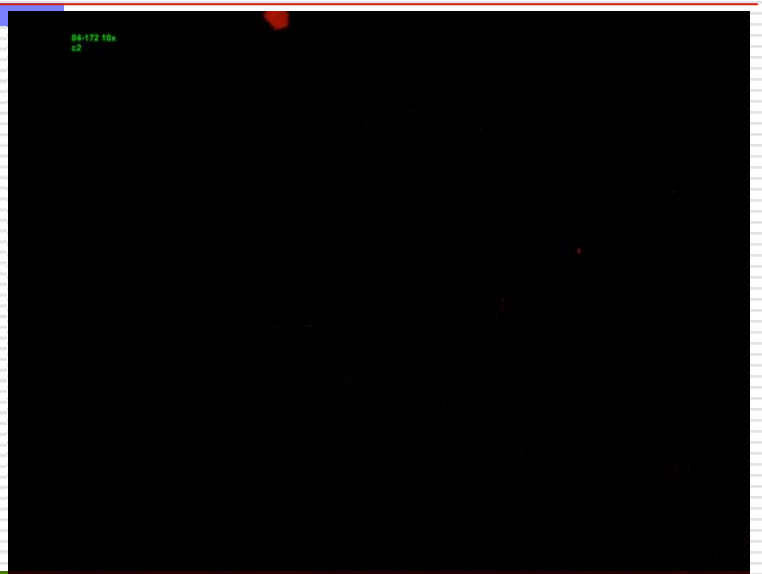


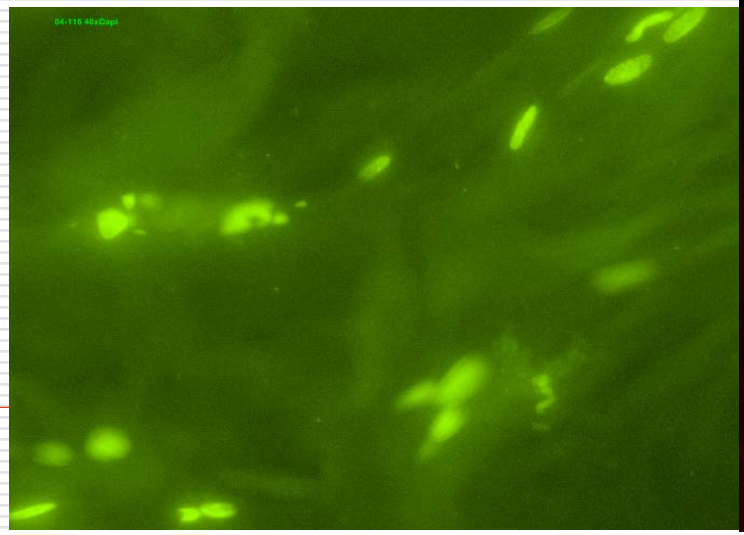
TC6, Ni6
WL6, WH6

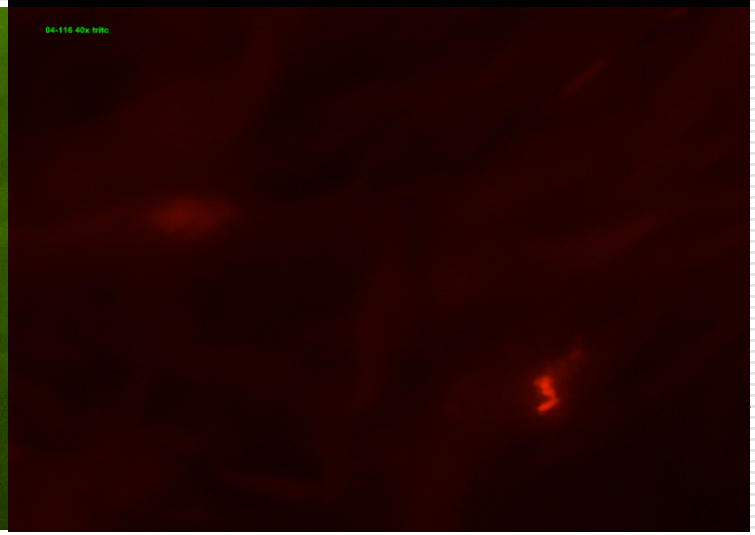


Apoptosis

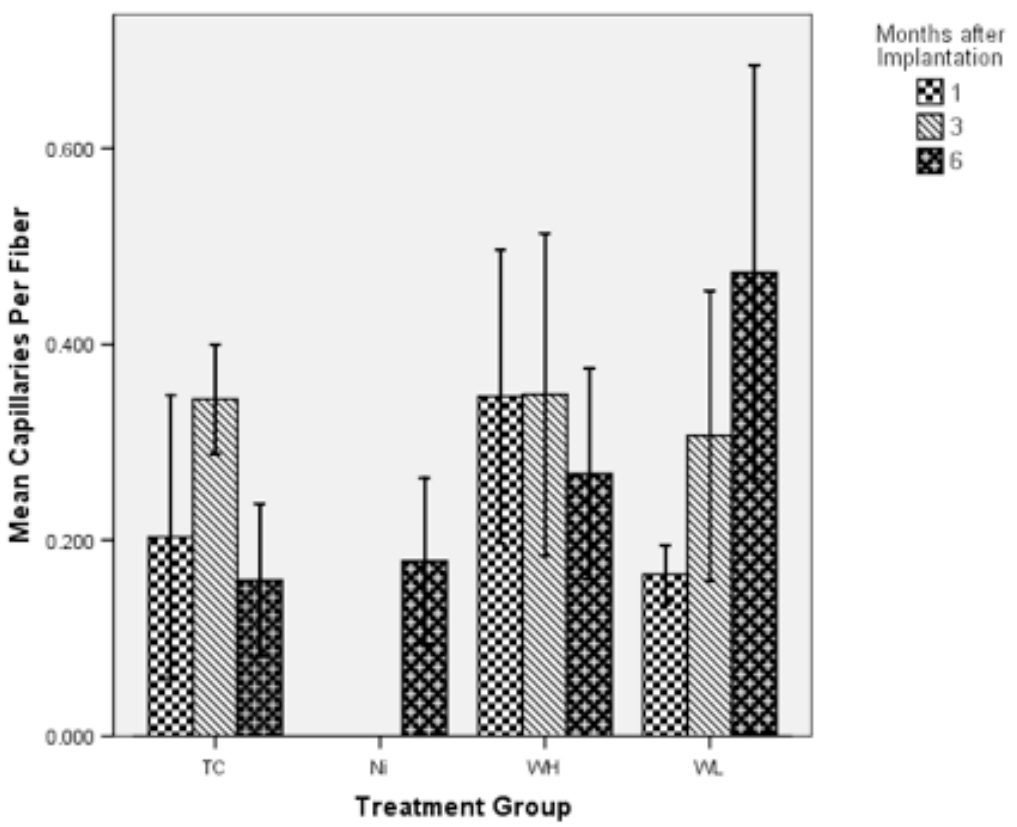

3.5
3
2.5
2
1.5
1
0.5
0
ApopTag Red Positive Cells (%)
1
3
6
Groups by Time after Implantation
TC
Ni
WH
WL







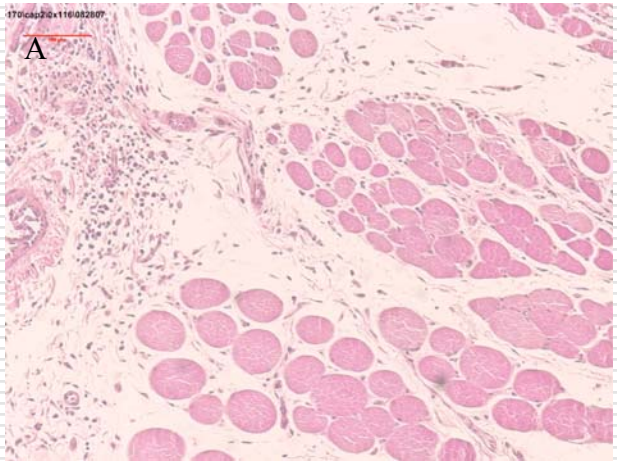
Effects of Embedded Metals on Vascularity

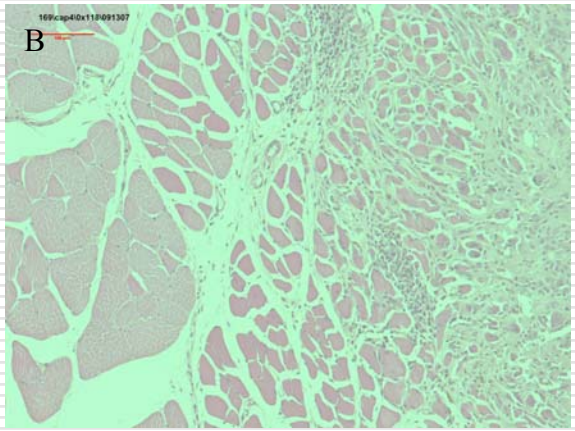


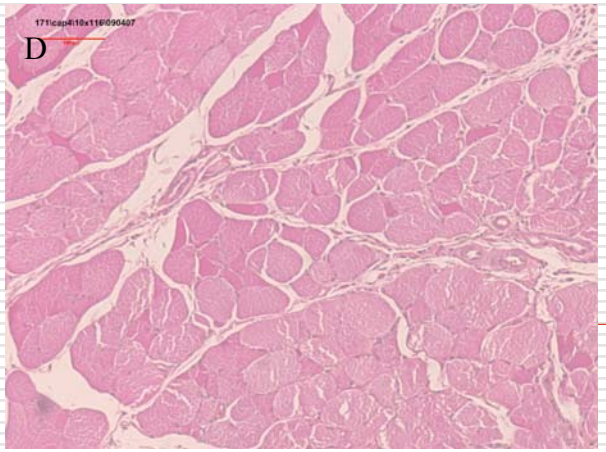
Selected Histological Parameters for Rats Implanted with Heavy Metals

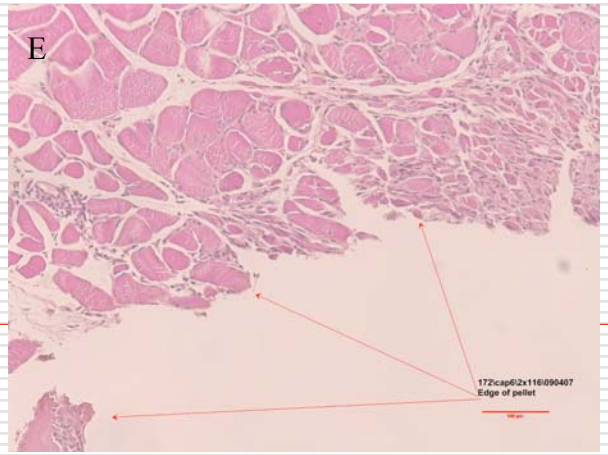
	Months after Surgery	Ta		WA (low)		WA (high)	
Neoplastic area (μm^2)	1	0	± 0	902000	± 492323	4301829	± 1915685
	3	0	± 0	1200518	± 594398.0	5875920	± 4069301
	6	0	± 0	2E+007	± 8794823	5E+007	± 2E+007
Mitotic Figures	1	0	± 0	13.00	± 7.22	2.50	± 1.20
	3	0	± 0	18.50	± 8.28	1.60	± 0.748
	6	0	± 0	21.83	± 8.27	8.5	± 2.60
Fiber size in μm^w	1	1575	± 191	1459	± 181	1747	± 426
	3	2667	± 327	1840	± 412	1968	± 279
	6	2122	± 445	1996	± 256	1767	± 486
Vessels per Fiber	1	0.2	± .07	0.16	± 0.02	0.35	± 0.07
	3	0.34	± .03	0.31	± 0.07	0.35	± 0.08
	6	0.16	± .04	0.47	± 0.11	0.27	± 0.05
Disintegration of Fibers (%)	1	0	± 0	11.02	± 6.72	17.89	± 16.44
	3	0	± 0	28.61	± 5.72	69.49	± 18.04
	6	0	± 0	22.81	± 7.09	77.82	± 10.95
Nuclei per Fiber	1	1.3	± 0.09	0.97	± 0.15	0.93	± 0.24
	3	0.98	± 0.11	1.45	± 0.33	0.61	± 0.28
	6	0.93	± 0.76	1.75	± 0.21	1.47	± 0.28
Circularity (1 = perfect circle)	1	0.79	± 0.003	0.76	± 0.003	0.73	± 0.003
	3	0.70	± 0.003	0.74	± 0.003	0.77	± 0.003
	6	0.72	± 0.004	0.75	± 0.003	0.76	± 0.003
Weight at Euthanasia (g)	1	267.77	± 7.36	300.23	± 5.83	287.12	± 7.49
	3	392.18	± 9.38	354.90	± 11.76	365.52	± 16.72
	6	454.23	± 7.19	476.25	± 10.51	419.57	± 11.15

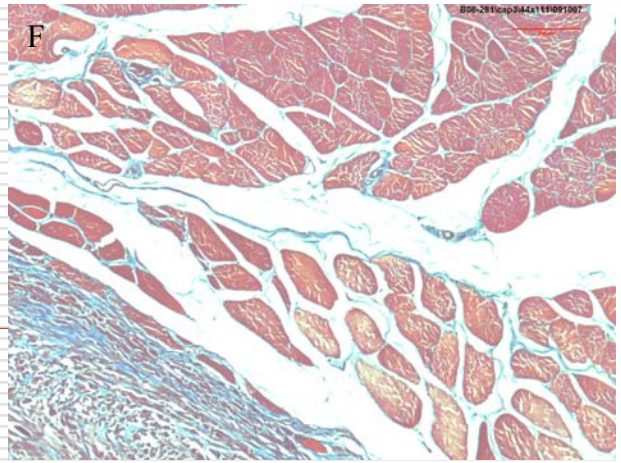
Muscle Fiber Changes



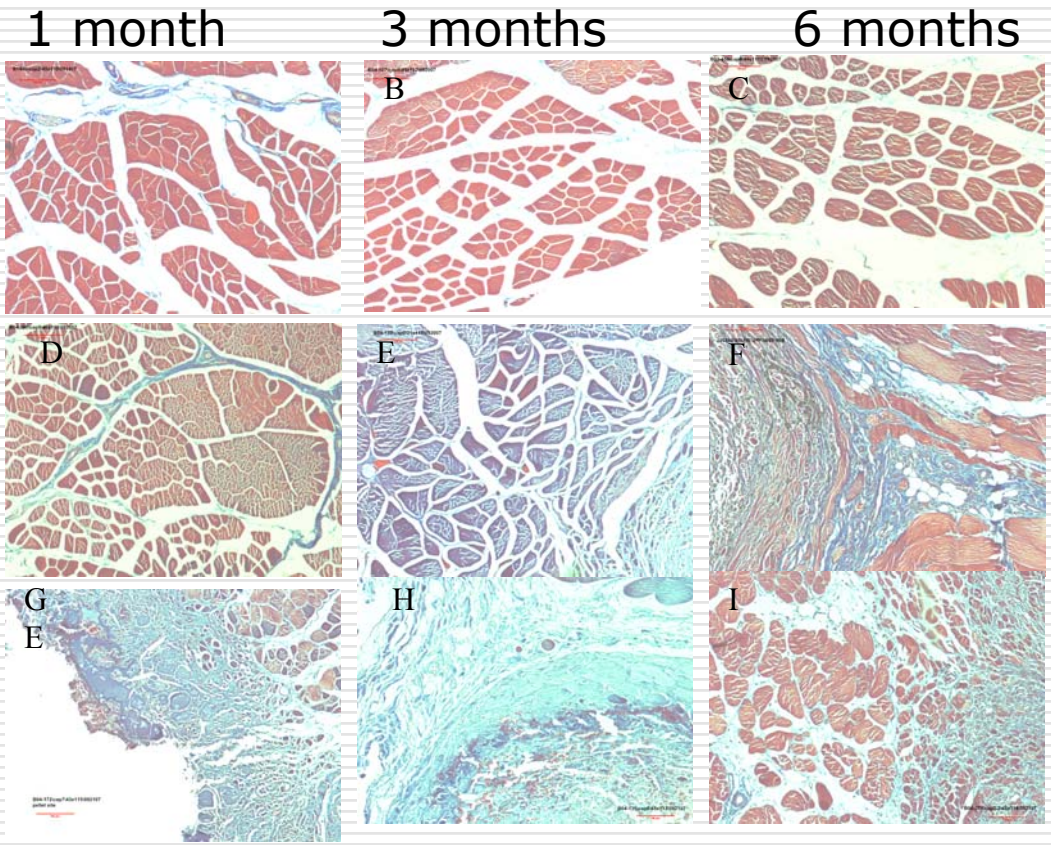




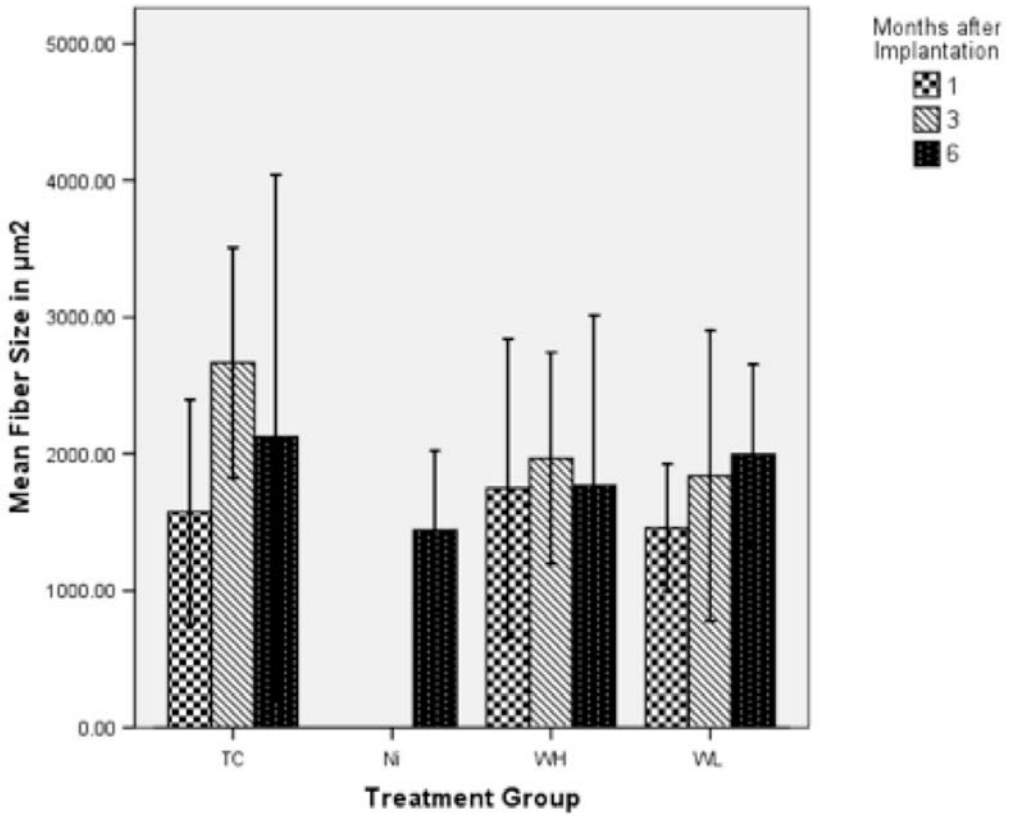




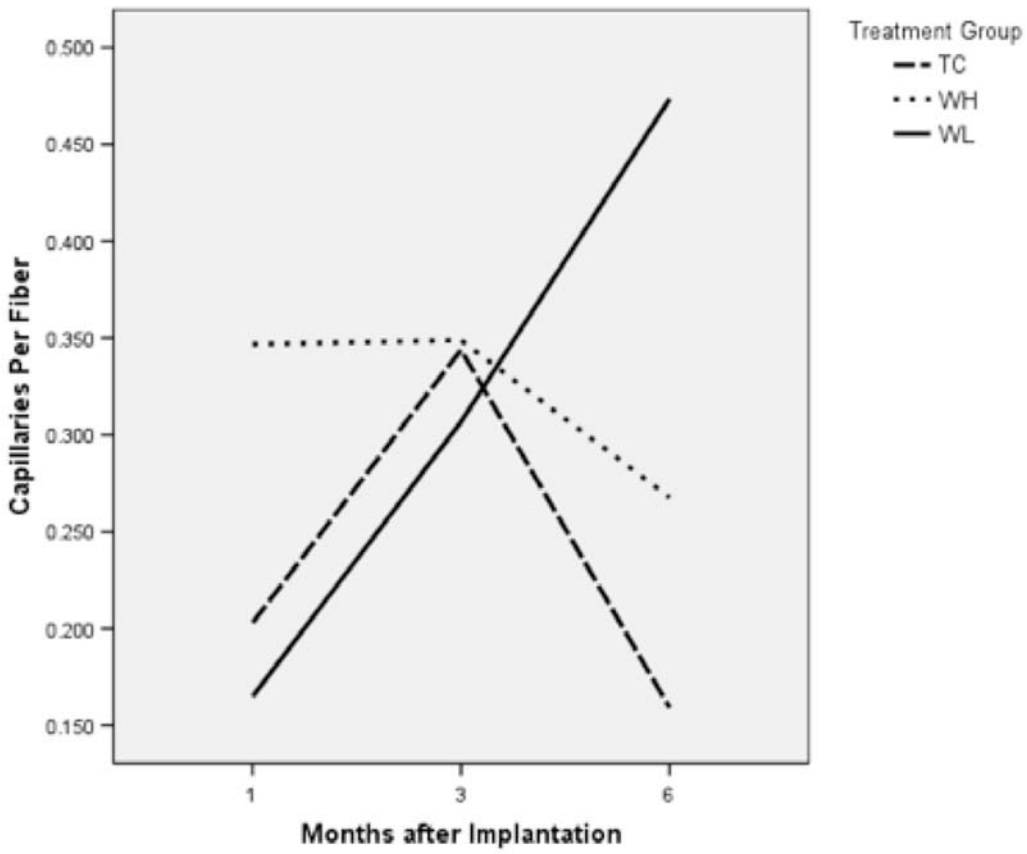
Collagen Formation in Rats with Embedded Ta and WA



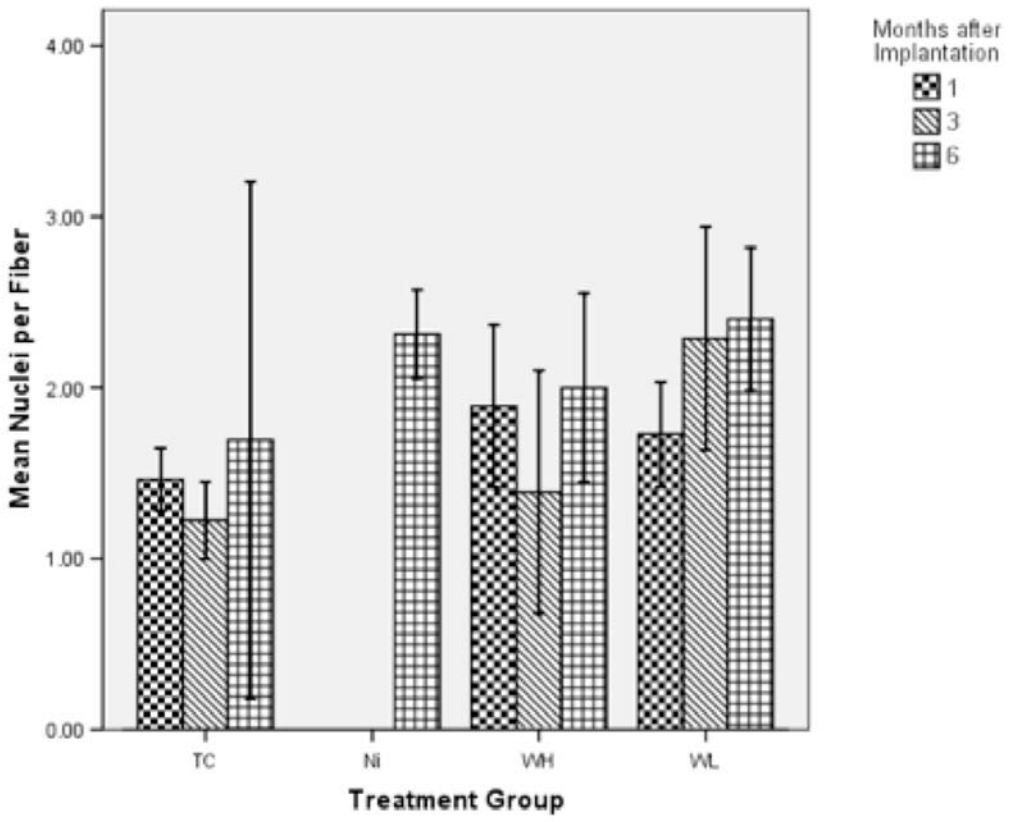
Mean Fiber Size in μm2 for all Rats

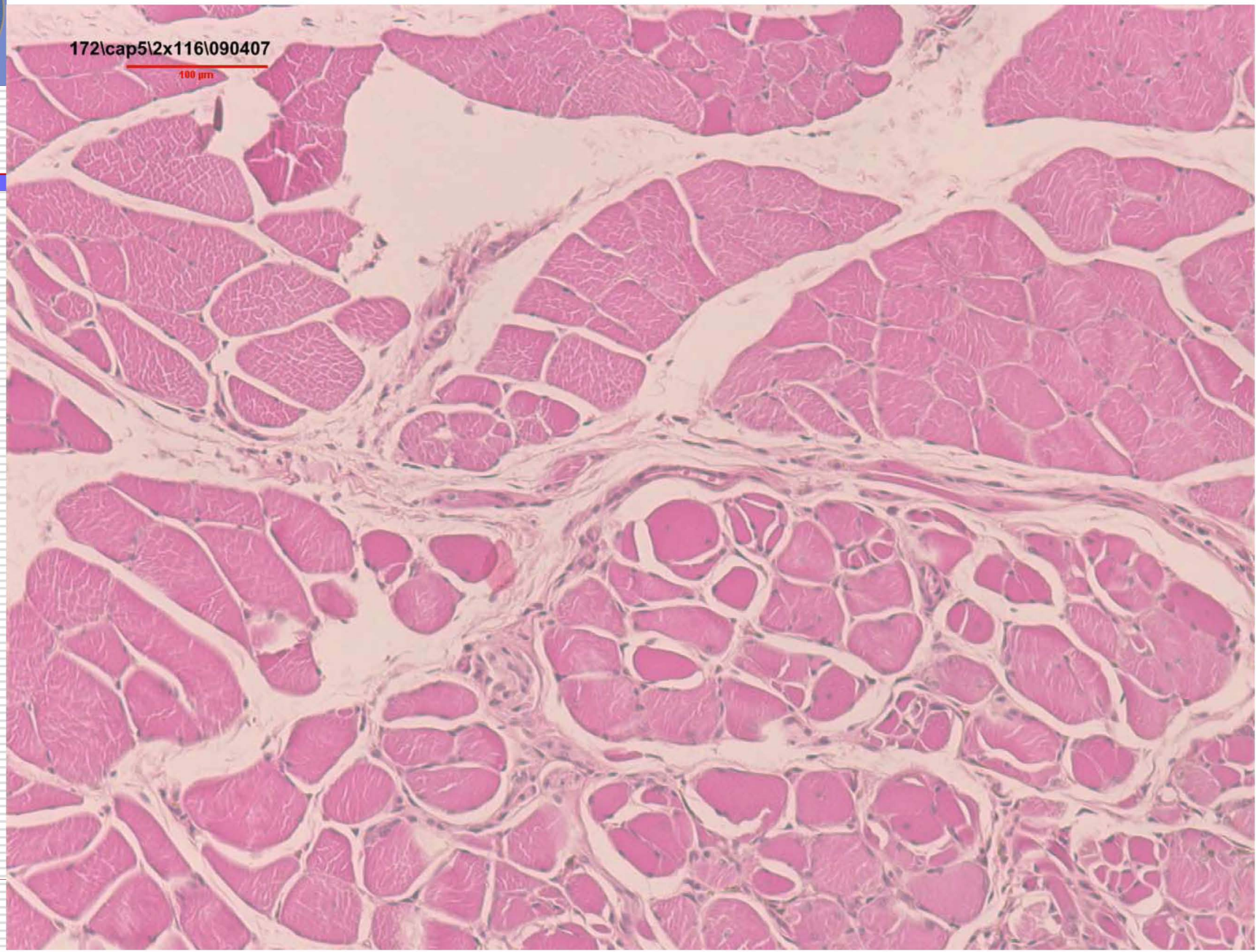


Capillaries per Fiber



Nuclei per Fiber




172\cap5\2x116\090407
100 µm

Significant Findings

- The six major findings from this study are
  - atrophy as early as 1-month post implantation,
  - neoplastic changes as early as 1-month post implantation,
  - a marked destruction of myofibers starting at 1-month post-implantation,
  - an increase in collagen spreading outward from the pellet implantation site as early as 1-month post implantation,
  - Increase in mitotic figures, and
  - Apoptosis

Conclusions

- Despite a receiving a lower dose, the WA low-dose group developed a tumor that has more mitotic figures, greater vascularity, and more apoptotic bodies than those in the WA high-dose group
- It is possible that the higher overall localized concentration of Ni and Co in the WA high-dose group had a toxic effect on the surrounding tissue
- This would explain the early capillary development followed by a decrease in capillaries and less necrosis.
- These locally high metal concentrations may cause immediate cell death in the WA high, whereas in the tungsten low lower metal concentrations and therefore less localized toxicity resulted in higher prognostic factors for RMS & > genotoxicity
- This would be consistent with the findings of Lehnert and explain why there is greater apoptosis and more mitotic figures with the WA low dose (the “bystander effect”)

Conclusions

- Environmentally, when tungsten is mixed with soil at rates greater than 1%, it results in microbial changes.
  - Loss of bacterial components, fungal biomass is increased and red worms die
  - U.S. does not regulate tungsten. However, the Russian Federation does regulate with a limit of 0.05mg l^{-1} in drinking water and 0.0008 mg l^{-1} in lakes
  - Recently, there has been more interest in the U.S. after an investigation of a leukemia cluster in children in Fallon, Nevada by the Centers for Disease Control and Prevention (CDC) and various state agencies

Conclusions

- This study raises concern about the potential health effects, including:
  - Carcinogenicity, of the tungsten alloys.
  - Standard surgical guidelines recommend leaving fragments in place
  - Shows that even at low doses this may not be a wise decision with embedded WA
  - While not definitive, suggests that regular monitoring of those with shrapnel injuries containing heavy metals may be warranted.

Future Research

- Longitudinal study
- Clinical care of patients with shrapnel injuries
- Rodent studies of other forms of shrapnel injuries and the histologic changes
- Policy analysis

© (1971/72); Friedensreich Hundertwasser

Uniformed Services University of the Health Sciences
Graduate School of Nursing
PhD Program
Verification of Completion of
Qualifying Examination
Form L

Verification of Submission of Qualifying Examination

Date: 4-10-

Dissertation Chairperson: Christine Tate **Date:** 4-10-08

Verification of PhD Student Passing Qualifying Examination

Date: 4-10-08

Dissertation Chairperson: Christine Tate **Date:** 4-10-08

(The verifications above can be notification by Email or Other Written Communication)

Verification of Successful Completion of Qualifying Examination

Attach Policy Statement from Appropriate Class Year in Handbook Regarding Requirement of Passing Grade on Qualifying Examination Prior to Dissertation Proposal Defense

Form E: Report of Proposal Defense Examination To Be Attached

Form L: Applicable to PhD Students Records from Inaugural to 2006 Entry Class
Supports Doctoral Handbook Policies Prior to the 2007 Handbook

Uniformed Services University of the Health Sciences
Graduate School of Nursing
PhD Program
Request for Dissertation Defense Date for the
Doctor of Philosophy Degree (Form F)

Name of Student: _____Roberta P. Lavin_____

Request for doctoral dissertation defense date of the student named above:__April 10, 2008____

The title of the dissertation is:___Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents_____

The Majority of the Dissertation Advisory Committee Members are available on this date:

Signature, Chairperson _Christine E. Kasper, PhD Printed Name (Yes) or No

Signature, Committee Member _John F. Kalinich, PhD_____ Printed Name (Yes) or No

Roopa Biswas
Signature, Committee Member _Roopa Biswas, PhD _____ Printed Name (Yes) or No

Marguerite L. Kearney
Signature, Committee Member _Marguerite L. Kearner, PhD Printed Name (Yes) or No

Approval/Disapproval

Signature: _____ Date: 4-10-08
Director, Doctoral Program

Approval/Disapproval

Signature: _____ Date: _____
Dean, Graduate School of Nursing, USUHS

Form F_A: Applicable to PhD Students Records from Inaugural to 2006 Entry Class
Supports Doctoral Handbook Policies Prior to the 2007 Handbook

Uniformed Services University of the Health Sciences
Graduate School of Nursing

Certification of Dissertation (Form I)

Name of Student: Roberta P. Lavin

This is to certify that the accompanying copies of the PhD dissertation of the student named above are completed and correct copies as approved by the Dissertation Advisory Committee.

Title of the
Dissertation: Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents

Signature, Chair | Christine E. Kasper (Printed Name) | 4-10-08 (Date)

Approval/Disapproval

Signature: _____ Date: 4-10-08
Director, PhD Program

Aproval/Disappproval

Signature: _____ Date: _____
Dean, Graduate School of Nursing, USUHS

Uniformed Services University of the Health Sciences
Graduate School of Nursing
Report of Dissertation Defense for the
Doctor of Philosophy Degree (Form H)

Title of the dissertation: ____ Histologic Changes as Indicators of Carcinogenicity of Tungsten Alloy in Rodents _____

_____

The decision of the Dissertation Committee is:

PASS
A. Both the dissertation and the oral defense are satisfactory: ✓
B. Minor changes are recommended by the Dissertation Advisory Committee that are to be made to the satisfaction of the Dissertation Chairperson: _____

DEFER
A. Major changes in the dissertation are required. Changes must be made to the satisfaction of the Dissertation Chairperson: _____
B. Major changes in the dissertation are required. Changes must be made to the satisfaction of the Dissertation Advisory Committee and at that time the oral defense will be rescheduled: _____

FAIL
Neither the oral performance nor the dissertation are adequate: _____

Signatures of the Committee
Chairperson: _____
Member: Marguerite L Kearney
Member: _____
Member: Roopa Biswas
Member: _____
Member: _____

Approval/Disapproval
Signature: _____ Date: 4-10-08
Director, Doctoral Program

Approval/Disapproval
Signature: _____ Date: _____
Dean, Graduate School of Nursing, USUHS

Form H_A: Applicable to PhD Students Records from Inaugural to 2006 Entry Class
Supports Doctoral Handbook Policies Prior to the 2007 Handbook

Reply Reply to all Forward | | | Close | Help

You replied on 11/5/2007 2:53 PM.

From: Barbara.StPierreSchneider@unlv.edu [Barbara.StPierreSchneider@unlv.edu] Sent: Mon 11/5/2007 11:26 AM

To: Lavin, Roberta (ACF); Lavin, Roberta (HHS/ASPR)

Cc: marnie.wiss@unlv.edu

Subject: Biological Research for Nursing - Decision on Manuscript ID BRN-RL-07-10-0045

Attachments:

View As Web Page

05-Nov-2007

Dear Ms. Lavin:

The review of your manuscript entitled "Effects of Heavy Metals and Ionizing Radiation on Skeletal Muscle: Shrapnel Injuries as a Modern Hazard" for the special issue, Skeletal Muscle Biology, is now complete. The comments of the reviewer(s) are included at the bottom of this letter. As you can see, the reviewers had numerous suggestions for improving your manuscript.

Therefore, the editorial decision is to ask you to revise and resubmit the manuscript. While I would not ask you to do this if I did not think it had a good chance of being accepted for publication, I cannot guarantee acceptance at this time. I recommend that you carefully read the comments and determine if you are able to address the concerns. If so, I encourage you to revise and resubmit the paper.

Please submit the revised version of your manuscript within the next 4 weeks. If we do not receive it within this timeframe, we cannot guarantee that we will be able to consider it for inclusion in the special issue. We will, however, consider it for a general issue of BRN. If we do not receive the revised manuscript within 6 months, it will be considered a new submission and will lose priority in our publication queue.

To revise your manuscript, log into http://mc.manuscriptcentral.com/brn and enter your Author Center. You will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number will be appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. You will be asked to upload the revised manuscript during the course of the revision-creation process.

When submitting your revised manuscript, please summarize the major changes you have made in the space provided. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

In closing I want to thank you and your colleagues for allowing us to consider your manuscript. Whether you decide to revise and resubmit your manuscript here or elsewhere, I hope that you will find the comments helpful. I also hope that you will continue to keep Biological Research for Nursing in mind as you prepare other manuscripts for publication consideration

Sincerely,
Barbara St. Pierre Schneider, DNSc, RN
Guest Editor, Biological Research for Nursing
Associate Dean for Research
School of Nursing
[redacted]5 Maryland Pkwy, Box 453018

Lavin, Roberta (ACF)

From: Nursing Outlook [nursingoutlook@aol.com]
Sent: Tuesday, March 18, 2008 11:07 PM
To: Lavin, Roberta (ACF)
Subject: A manuscript number has been assigned: NO-492

Ms. Ref. No.: NO-492
Title: Treatment of Victims of Violence: From the Frontlines to City Streets Nursing Outlook

Dear CAPT Roberta Lavin,

Your submission entitled "Treatment of Victims of Violence: From the Frontlines to City Streets" has been been assigned the following manuscript number: NO-492.

You may check on the progress of your paper by logging on to the Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/ymno/.
Your username is: rlavin10
Your password is: lavin768676
Thank you for submitting your work to this journal.

Kind regards,

Julia Snethen
Editorial Assistant
Nursing Outlook

Subject: CANCER RESEARCH CAN-08-1206 -- Receipt of New Manuscript
Date: Tuesday, April 1, 2008 3:54 PM
From: nicole.foulke@aacr.org
To: roberta.lavin@acf.hhs.gov
Cc: rlavin10@mac.com
Conversation: CANCER RESEARCH CAN-08-1206 -- Receipt of New Manuscript

Dear Ms. Lavin,

You have successfully submitted your manuscript to CANCER RESEARCH. Your manuscript information is as follows:

MANUSCRIPT NUMBER
CAN-08-1206

MANUSCRIPT TITLE
Carcinogenicity of Embedded Tungsten Alloy in Rodents

Please take note of this information for future reference and refer to the manuscript number should you need to contact the journal office during the review process.

You may check on the status of this manuscript by selecting the "Check Manuscript Status" link below:
http://can.msubmit.net/cgi-bin/main.plex?el=A2Cs3EIM5A5fie6F3A9AjrN4KaVuphCpfZLt1pkQZ

(Press/Click on the above link to be automatically sent to the web page.)

Thank you for submitting your work to CANCER RESEARCH.

Sincerely,
CANCER RESEARCH

Subject: The Journal of Physiology - Manuscript Submission
Date: Friday, April 4, 2008 6:04 PM
From: journals@physoc.org
To: "Lavin, Roberta (ACF)" roberta.lavin@acf.hhs.gov
Conversation: The Journal of Physiology - Manuscript Submission

JPHYSIOL/2008/154831
"Skeletal Muscle Damage as an Early Indicator of Adverse Health Effects of Tungsten Alloy"
by Roberta P Lavin, John F. Kalinich, Marguerite L. Kearney, and Christine E. Kasper

This is an automatic message acknowledging your online submission to

The Journal of Physiology

Enclosure 3

Uniformed Services University
of the Health Sciences

Manuscript Approval or Clearance*

INITIATOR

1. USU Principal author: Roberta Lavin
2. Academic title: PhD Candidate
3. School/Department: GSN
4. Phone: 202-369-7602
5. Type of publication (submitted to): Paper ☐ Article ☒ Book ☐
   USU WWW Home Page at (location) _____
   Other: _____
6. Manuscript title: Effects of Heavy metals on Skeletal muscle: Potential Targets for Nursing
7. Intended publication (include organization if appropriate): Biological Research in Nursing
8. Required by (publication receipt) date: 9/30/07
9. Date submitted for USU approval: 8/19/07

CHAIR OR DEPARTMENT HEAD APPROVAL

1. Name: Christine E. Kasper, PhD, RN
2. School/Department: GSN, Doctoral Program
3. Date: March 20, 2008
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☒ Chair or Department Head Approval Christine E Kasper

Chair or Department Head Signature/Date

(If additional approval or clearance is required, see other side of form)

USUHS Form 5230 (VAM) (10/99)

Comments:
1. No academic credentials associated w/ Primary Author
2. Identify GSN as academic affiliate along w/ USU
~~3. No disclaimer statement~~
4. Dates 9/07?

Enclosure 3

DEAN APPROVAL

1. Name: _____
2. School/Department: Graduate School of Nursing
3. Date: _____
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

~~☐ Dean Approval~~ note: I cannot approve or disapprove since it is already submitted.

Susan Scholar 3/24/08
Dean Signature/Date

DIRECTOR, UNIVERSITY AFFAIRS (OUA) ACTION

1. Name: _____
2. Date: _____
3. ☐ USU Approved or
   ☐ DoD approval/clearance required
4. ☐ Submitted to DoD (Health Affairs) on (date): _____
   or
   ☐ Submitted to DoD (Public Affairs) on (date): _____
5. DoD approved/cleared (as written) or ☐ DoD approved/cleared (with changes)
6. DoD clearance/date: _____
7. DoD disapproval/date: _____

Carol Schim
Director, OUA Signature/Date

Enclosure 3

Uniformed Services University
of the Health Sciences

Manuscript Approval or Clearance*

INITIATOR

1. USU Principal author: CAPT Roberta Lavin
2. Academic title: PhD Candidate
3. School/Department: GSN
4. Phone: 202-401-9306
5. Type of publication (submitted to): Paper ☐ Article ☑ Book ☐
   USU WWW Home Page at (location) _____
   Other: _____
6. Manuscript title: Treatment of Victims of Violence: From Front lines to City Streets
7. Intended publication (include organization if appropriate): Nursing Outlook
8. Required by (publication receipt) date: _____
9. Date submitted for USU approval: 3/10/08

CHAIR OR DEPARTMENT HEAD APPROVAL

1. Name: Christine E. Kasper
2. School/Department: USUHS / GSN
3. Date: 3/10/08
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *__Note:__ *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☑ Chair or Department Head Approval

Christine Kasper
Chair or Department Head Signature/Date 3/10/08

(If additional approval or clearance is required, see other side of form)

USUHS Form 5230 (VAM) (10/99)

DEAN APPROVAL

1. Name: _____
2. School/Department: Graduate School of Nursing
3. Date: _____
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☑ Dean Approval

Comments: ① please i.d. the GSN along c USU on title page
② No academic credentials associated c Author

Dean Signature/Date 3/24/08

DIRECTOR, UNIVERSITY AFFAIRS (OUA) ACTION

1. Name: _____
2. Date: _____
3. ☐ USU Approved or
   ☐ DoD approval/clearance required
4. ☐ Submitted to DoD (Health Affairs) on (date): _____
   or
   ☐ Submitted to DoD (Public Affairs) on (date): _____
5. DoD approved/cleared (as written) or ☐ DoD approved/cleared (with changes)
6. DoD clearance/date: _____
7. DoD disapproval/date: _____

Director, OUA Signature/Date

Enclosure 3

Uniformed Services University
of the Health Sciences

Manuscript Approval or Clearance*

AUTHOR

1. USU Principal author: Roberta Lavin
2. Academic title: PhD Candidate
3. School/Department: GSN
4. Phone: 202-365-7602
5. Type of publication (submitted to): Paper ☐ Article ☒ Book ☐
   USU WWW Home Page at (location) _____
   Other: _____
6. Manuscript title: Oncogenicity of Embedded Tungsten Alloy in Rodents
7. Intended publication (include organization if appropriate): Cancer Research
8. Required by (publication receipt) date: 3/24/08
9. Date submitted for USU approval: 3/7/08

CHAIR OR DEPARTMENT HEAD APPROVAL

1. Name: Christine E. Kasper, PhD, RN
2. School/Department: GSN, Doctoral Program
3. Date: March 21, 208
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☒ Chair or Department Head Approval Christine E Kasper

Chair or Department Head Signature/Date

(If additional approval or clearance is required, see other side of form)

USUHS Form 5230 (VAM) (10/99)

Enclosure 3

DEAN APPROVAL

1. Name: _____
2. School/Department: Graduate School of Nursing
3. Date: _____
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☑ Dean Approval

Comment: I would suggest that the School be identified as the Academic Affiliate with the Institution on the title page

Bruce Scharnberg 3/25/05
Dean Signature/Date

DIRECTOR, UNIVERSITY AFFAIRS (OUA) ACTION

1. Name: _____
2. Date: _____
3. ☐ USU Approved or
   ☐ DoD approval/clearance required
4. ☐ Submitted to DoD (Health Affairs) on (date): _____
   or
   ☐ Submitted to DoD (Public Affairs) on (date): _____
5. DoD approved/cleared (as written) or ☐ DoD approved/cleared (with changes)
6. DoD clearance/date: _____
7. DoD disapproval/date: _____

Carol Schumacher
Director, OUA Signature/Date

Enclosure 3

Uniformed Services University
of the Health Sciences

Manuscript Approval or Clearance*

INITIATOR

1. USU Principal author: Roberta Zarin
2. Academic title: PhD Candidate
3. School/Department: GSN
4. Phone: 202-319-7602
5. Type of publication (submitted to): Paper ☐ Article ☒ Book ☐
   USU WWW Home Page at (location) _____
   Other: _____
6. Manuscript title: Skeletal Muscle Damage as an Early Indicator of Adverse Health Effects of Tungsten Alloy
7. Intended publication (include organization if appropriate): Journal of Physiology
8. Required by (publication receipt) date: _____
9. Date submitted for USU approval: 4/2/08

CHAIR OR DEPARTMENT HEAD APPROVAL

1. Name: Christine E. Kasper
2. School/Department: GSN
3. Date: 4/2/08
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *Note: *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☒ Chair or Department Head Approval

Christine E. Kasper 4/1/08
Chair or Department Head Signature/Date

(If additional approval or clearance is required, see other side of form)

USUHS Form 5230 (VAM) (10/99)

Enclosure 3

DEAN APPROVAL

1. Name: _____
2. School/Department: _____
3. Date: _____
4. ☐ Higher approval/clearance required (for University-, DoD or U.S. Government-level policy, communication systems or weapons issues review*).
   *__Note:__ *It is DoD policy that clearance of information or material shall be granted if classified areas are not jeopardized, and the author accurately portrays official policy, even if the author takes issue with that policy. Material officially representing the view or position of the University, DoD, or the Government is subject to editing or modification by the appropriate approving authority.*

☑ Dean Approval

Bruce Schumm 4/2/08
Dean Signature/Date

DIRECTOR, UNIVERSITY AFFAIRS (OUA) ACTION

1. Name: _____
2. Date: _____
3. ☐ USU Approved or
   ☐ DoD approval/clearance required
4. ☐ Submitted to DoD (Health Affairs) on (date): _____
   or
   ☐ Submitted to DoD (Public Affairs) on (date): _____
5. DoD approved/cleared (as written) or ☐ DoD approved/cleared (with changes)
6. DoD clearance/date: _____
7. DoD disapproval/date: _____

Thomas E. Ward 4/3/08
Director, OUA Signature/Date

Abstract: Submitted to Force Health Protection Conference, Albuquerque, New Mexico

The long-term goal of *in situ* shrapnel identification is to give the patient treatment options that will result in the best outcomes. Each person will differ in his or her existing health status, degree of shrapnel injury, and ability to return to optimal health. There has been evidence that even shrapnel injuries not involving heavy metal tungsten alloy (HMTA) can result in tumor formation and other long-term health consequences. The indicators of these long-term adverse health effects have not been identified. Data presented from this research study will provide indications of early signs of skeletal muscle changes that may indicate impending adverse consequences of embedded HMTA. The central hypothesis of the research is that there is a significant difference between the magnitude of skeletal muscle damage in F344 rats embedded with HMTA and those embedded with either positive (Ni) or negative (Ta) controls.

Subject: Poster Acceptance (UNCLASSIFIED)
Date: Thursday, April 3, 2008 8:45 AM
From: Gibson, Annemarie Ms USACHPPM <annemarie.gibson@us.army.mil>
To: "Lavin, Roberta (ACF)" roberta.lavin@acf.hhs.gov
Cc: "Gibson, Annemarie Ms USACHPPM" annemarie.gibson@us.army.mil
Conversation: Poster Acceptance (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

CONGRATULATIONS. Your poster (Shrapnel Injuries: What Nurses Should Monitor Now and Long-term) has been accepted for display at the 11th Annual Force Health Protection Conference in Albuquerque, New Mexico, 9-16 August 2008.

BY 1 JULY 2008: Please confirm your intent to display your poster by sending an e-mail message to annemarie.gibson@us.army.mil. If needed, please update the title of your poster and the primary author's name.

INSTRUCTIONS
1. You will have a 4' height and 8' width of display space on the poster
stand. The poster stand will accept Velcro, thumb tacks, and push pins. The poster stands are two-sided (separate posters on either side) and have metal-leg supports on either end. These ARE NOT tabletop displays. If you want to have business cards or handouts available, please configure a dispensing or holding container as part of your display.

2. I anticipate 80-100 posters on a variety of topics. To attract the attention of the audience, I encourage you to keep the text simple and rely on pictures, graphs, and bullet topics to present your message. Also, if you have access to a plotter, plotter paper makes a light-weight, easy-to-hang poster. If you want to laminate your poster, please use a very thin laminate that Velcro and push pins can support. Heavy posters will be very difficult to secure.

3. All poster submissions should be OPSEC and/or PAO reviewed prior to the conference to ensure your poster meets public release requirements. This should be done at your command level.

4. Please label your poster tube or case with your name, address, and phone number. Storage is available in the registration area in the Lobby outside the exhibit hall on the east side of the convention center, ground level. Please label the back of your poster with name and contact information of the individual who will remove or claim the poster.

5. Posters will be displayed on the west side of the convention center in the atrium at the foot of the escalators. You will be required to bring your poster to the Poster Check-In table and get a poster number in advance of the conference. This number will serve to identify your poster for display location and judging. ALL POSTERS MUST BE CHECKED IN AT THE REGISTRATION AREA PRIOR TO POSTING.

6. Hours:
a. Set-up. Monday 11 August, 1500-1900 hours; Tuesday 12 August between 0800-1000 hours. (Poster stands are erected late on the first day; please be patient.)
b. Poster Staffing hours: Tuesday, 12August, 1100-1300 hours. (The only time you must attend your poster). Military may wear ACUs or standard duty uniform.
c. Poster Viewing hours: Tuesday, 12 August, through Thursday, 14 August, 0800-1700 hours. Friday, 15 August - 0800-1100.
d. Poster Removal: 1100 hours Friday, 15 August. The exhibits and poster stands come down immediately after the exhibits close, POSTERS NOT REMOVED WILL BE MOVED TO THE REGISTRATION DESK where they may be claimed. Posters not claimed by 1300 hours, Friday, 15 August, will be discarded.

7. Posters will be judged in four separate categories (Clinical, Operational, Health Promotion and Wellness, and Research), each for technical content, presentation and aesthetics by a peer panel of subject matter experts. Winners will be recognized at the Plenary Session on Thursday, 14 August.

8. Other important items.
a. Chairs may not be readily available, so you may have to stand on your feet during the staffing hours.
b. Posters will be listed in the conference agenda book. Listing is not guaranteed for posters received after 1 June 2008.
c. Please ensure the title of your poster is PROMINENTLY DISPLAYED. Author names and affiliations should also be included but may be more discrete.
d. Don't forget to label the back of your poster with your name, address, and phone number so that posters may be returned to the proper owner/agency.

9. Your on-site Poster POC is Anne Gibson, (cell: 410-322-9859 at the conference only). If you have any questions or concerns, please contact Anne at 410-436-3254, DSN 584-3254 or FAX: 410-436-1039.

ANNE GIBSON
Chief, Publication Management Division

Deputy Chief of Staff for Communication
U.S. Ctr for Health Promo & Prev Med
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403
PH: 410-436-3254 DSN: 584-3254
FAX: 410-436-1039
email: annemarie.gibson@us.army.mil
Classification: UNCLASSIFIED
Caveats: NONE

Publications While at the Uniformed Services University of the Health Sciences, Graduate School of Nursing

PUBLICATIONS

Author	(in press)	Perekev, M., Hsu, S., **Lavin, R.** Healthcare Facilities Sustainability – Long-term Issures. In R. Powers (ed.). *International disaster Nursing,* Madison, WI: World Association for Disaster and Emergency Medicine.
	2007	**Lavin, R.,** Dreyfuss, M., Slepski, L., & Kasper, C. Subject Matter Experts: Fact or Fiction. *Nursing Forum, 42(4).*
	2007	**Lavin, R**., Harrington, M., Lavine, A., Agbot-Tabi, E. & Erger, N. Critical Infrastructure Protection: Why Physicians, Nurses, and Other Healthcare Professional Need to be Involved. *American Journal of Disaster Medicine,* 1(1), p 48 - 54.
	2007	Savitz, L., **Lavin, R**. & Root, E. (2007). Geographical Information Systems as a Strategic Tool for Better Planning, Response, and Recovery. In *National Security Issues in Science, Law, and Technology.* Taylor & Francis, Boca Raton.
	2007	**Lavin, R**., Slepski, L., & Veenema, T. Leadership and Coordination in Disaster Health Care Systems: The Federal Disaster Response Network. In Veenema, T. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.
	2007	Riccardi, R., Agazio, J., **Lavin, R**., & Walker, P. Directions in Nursing Research and Development. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.
	2007	**Lavin, R.** & Dreyfuss, M. HIPAA Case Study. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards,* 2nd Ed., New York, Springer Publishing Co.
	2006	**Reeves, G.,** Lavin, R**.,** & Slepski, L. *Online Preparedness Education Program*. *Radiation Dispersal Device.* Available at http://opep.usuhs.edu/overview_acknow.do**.**
	2006	**Lavin, R.** HIPAA and Disaster Research: Preparing to Conduct Research. *Disaster Management and Response,* 4(2), 32-37.
	2006	Chaffee, M., **Lavin, R.** & Slepski, L. Nursing Role: Homeland Security. *Current Issues in Nursing*, 7th Ed. Mosby, St. Louis, MO.
	2005	Couig, M., Martinelli, A., & **Lavin, R.** The National Response Plan: Health and Human Services the Lead for Emergency Support Function #8. *Disaster Management & Response,* 3(2), 34-40.
	2004	Phillips, S. & **Lavin, R.** Readiness and Response to Public Health Emergencies. *Journal of Professional Nursing*. November, 2004.

Significant Work and Awards While Attending the Uniformed Services University for the Health Sciences, Graduate School of Nursing

GUEST LECTURES

September 2007	**Lavin, R.** Incident Command System. Johns Hopkins University School of Nursing – Emergency Preparedness and Disaster Response Institute. Baltimore, MD.
September 2007	**Lavin, R.** National Critical Incident Response. Johns Hopkins University School of Nursing – Emergency Preparedness and Disaster Response Institute. Baltimore, MD.
August 2007	**Lavin, R.** Economic Consequences of Disaster. Homeland Security Nursing PhD Program. University of Tennessee College of Nursing, Knoxville, TN.
September 2006	**Lavin, R**. National Infrastructure and Components. Concepts and Principles of Disaster Preparedness, Management and Response. Johns Hopkins University School of Nursing, Baltimore, MD.
July 2006	**Lavin, R.** National Critical Incident Response. Johns Hopkins University School of Nursing – Emergency Preparedness and Disaster Response Institute. Baltimore, MD.
June 2006	**Lavin, R.** Economic Consequences of Disaster. Homeland Security Nursing PhD Program. University of Tennessee College of Nursing, Knoxville, TN.
January 2006	**Lavin, R.** Operational Readiness: Starting from Civil Defense. First year master's students. Uniformed Services University for the Health Sciences, Bethesda, MD.
January 2006	**Lavin, R.** Civil Defense History. Homeland Security Nursing PhD Program. University of Tennessee College of Nursing, Knoxville, TN.
September 2005	**Lavin, R.** National Infrastructure and Components. Concepts and Principles of Disaster Preparedness, Management and Response. Johns Hopkins University School of Nursing, Baltimore, MD.

PRESENTATIONS

November, 2007	Hus, S., **Lavin, R**., Borden, C. & Bardack, S. Burn Casualty Preparedness: A Program Evaluation of the Burn Asset Resource Tracking System. APHA 135 Annual Meeting and Expo. Washington, DC.
February 2007	**Lavin, R**., Lavine, A, & Agbor-Tabi, E. Critical Infrastructure Data System. Public Health Preparedness Summitt. Washington, DC.
June 2005	**Lavin, R.** Public Health Credentialing. Commissioned Officer's Association. Philadelphia, PA.
June 2005	**Lavin, R.** & Slepski, L. Poster - The International Nursing Preparedness Community: Roles. Commissioned Officer's Association, Philadelphia, PA.
April 2005	Gebbie, K. & **Lavin, R.** Emergency Preparedness Research Needs. Eastern Nursing Research Society. New York, NY.
April 2005	**Lavin, R.** Collaboration of HHS with the ABA. ABA's Leadership Conference. Washington, DC.

INVITED PRESENTATIONS

November 2004	**Lavin, R.** Sector Specific Agency Panel. Information Sharing and Analysis Council. George Mason University, Arlington, VA.
October 2007	**Lavin, R**. Leadership in Federal Disaster Response. C.J. Ready Army Nurse Leadership Conference. Arlington, VA.
October 2007	**Lavin, R**. Keynote: Pandemic Influenza Planning. Chico Kappa Omicron Research Conference: 21st Century Nursing. Chico, CA.
July 2007	Gibson, L. & **Lavin, R.** Case Management. IA ESF-6 Partners Conference. Baltimore, MD
May, 2007	**Lavin, R.** Keynote: Disaster Human Services Case Management. Regional Emergency Preparedness Conference. Atlanta, GA.
May 2007	**Lavin, R.** Social Services Block Grants. Substance Abuse and Mental Health Emergency Preparedness. New Orleans, LA.
July 2006	**Lavin, R.** Keynote: Frontiers in Biosurveillance. UT Alumni Summer College. Knoxville, TN.
June 2006	**Lavin, R.** Disaster Preparedness Training. Institute of Medicine. Washington, DC.
June 2006	**Lavin, R.** Pandemic Influenza: The Role of the Private Sector. Health Industry Distributors Association Conference. Arlington, VA.
May 2006	**Lavin, R.** Public Health Preparedness: Nurses' Critical Role. 2006 USPHS Professional Conference. Denver, CO.
April 2006	**Lavin, R.** Nursing Leadership in Combating Bioterrorism. RWJ Executive Nurse Fellows Program
April 2006	**Lavin, R.** Keynote: The National Perspective on Emergency Preparedness: Opportunities for ASTDN Linkage and Leadership. Association of State and Territorial Directors of Nursing. Seattle, WA.
March 2006	**Lavin, R.** Pandemic Influenza Preparedness Planning. California Association for Nurse Practitioners 29th Annual Education Conference. San Diego, CA.
February 2006	**Lavin, R.** & Anderson, M. Federal Disaster Resource and Updates Panel. HRSA Annual State Trauma Leadership Meeting. Arlington, VA.
November 2005	**Lavin, R**. Pandemic Influenza and Public Health Preparedness after Hurricane Katrina. AANP Leadership Meeting. Washington, DC
September 2005	**Lavin, R.** & Yeskey, K. Grand Rounds: Hurricane Katrina and Rita Response and the Future of Domestic Disaster Preparedness. Uniformed Services University for the Health Sciences, Bethesda, MD.

PUBLICATIONS

Author

(in press)	Perekev, M., Hsu, S., **Lavin, R.** Healthcare Facilities Sustainability – Long-term Issures. In R. Powers (ed.). *International disaster Nursing,* Madison, WI: World Association for Disaster and Emergency Medicine.
2007	**Lavin, R.,** Dreyfuss, M., Slepski, L., & Kasper, C. Subject Matter Experts: Fact or Fiction. *Nursing Forum, 42(4).*
2007	**Lavin, R**., Harrington, M., Lavine, A., Agbot-Tabi, E. & Erger, N. Critical Infrastructure Protection: Why Physicians, Nurses, and Other Healthcare Professional Need to be Involved. *American Journal of Disaster Medicine,* 1(1), p 48 - 54.

2007	Savitz, L., **Lavin, R**. & Root, E. (2007). Geographical Information Systems as a Strategic Tool for Better Planning, Response, and Recovery. In *National Security Issues in Science, Law, and Technology.* Taylor & Francis, Boca Raton.
2007	**Lavin, R**., Slepski, L., & Veenema, T. Leadership and Coordination in Disaster Health Care Systems: The Federal Disaster Response Network. In Veenema, T. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.
2007	Riccardi, R., Agazio, J., **Lavin, R**., & Walker, P. Directions in Nursing Research and Development. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards*, 2nd Ed., New York, Springer Publishing Co.
2007	**Lavin, R.** & Dreyfuss, M. HIPAA Case Study. *Disaster Nursing in Emergency Preparedness for Chemical, Biological, Radiological Terrorism and Other Hazards,* 2nd Ed., New York, Springer Publishing Co.
2006	**Reeves, G.,** Lavin, R**.,** & Slepski, L. *Online Preparedness Education Program*. *Radiation Dispersal Device.* Available at http://opep.usuhs.edu/overview_acknow.do**.**
2006	**Lavin, R.** HIPAA and Disaster Research: Preparing to Conduct Research. *Disaster Management and Response,* 4(2), 32-37.
2006	Chaffee, M., **Lavin, R.** & Slepski, L. Nursing Role: Homeland Security. *Current Issues in Nursing*, 7th Ed. Mosby, St. Louis, MO.
2005	Couig, M., Martinelli, A., & **Lavin, R.** The National Response Plan: Health and Human Services the Lead for Emergency Support Function #8. *Disaster Management & Response,* 3(2), 34-40.
2004	Phillips, S. & **Lavin, R.** Readiness and Response to Public Health Emergencies. *Journal of Professional Nursing*. November, 2004.

REVIEWER

Journals	2006 - present	♦ International Nursing Review
	2005 - present	♦ Journal of Specialist in Pediatric Nursing
	2005	♦ Radiological Acts of Terrorism: Are Nurses Ready? *Nursing Spectrum*

ADJUNCT FACULTY, CLINICAL TRAINING AGREEMENTS & PRECEPTOR		
Johns Hopkins University	FY07-09	• Adjunct Faculty in the School of Nursing • Preceptor to four MSN/MPH students
Loyola Hospital	FY07	• Clinical coordination of burn training for USPHS nurses and establishment of Memorandum of Agreement (MOA)
University of Tennessee	FY06	• Preceptor for PhD candidate for CIDS Analysis
Johns Hopkins University	FY06	• Preceptor for MSN/MPH student for burn bed tracking system
James Madison University	FY06	• Preceptor for undergraduate intern in National Defense
Arizona Burn Center at Maricopa Medical Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
Bridgeport Hospital Burn Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
The Burn Center at Washington Hospital Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
Regions Hospital Burn Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
Parkland Memorial Hospital Regional Burn Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
University of Washington Burn Center Harborview Medical Center	FY05-06	• Clinical coordination of burn training for USPHS nurses and establishment of MOA
FEDERALLY FUNDED PROJECT MANAGEMENT & RESEARCH		
$1M (combined ACF, ASPR, FEMA project)	FY08	• Research and development of the WFH Disaster Human Services Case Management pilot program
TAIC $550,000	FY08	• Human services support for regional and national planning and Secretary's Operations Center ESF-6 watch office support
TAIC $1.1M	FY07	• Human services regional disaster management support to meet the human services needs for experts in the regions
Dissertation $2,500	FY07-08	• USUHS dissertation funding

CIDS $1M (combined HHS & DHS study)	FY06-07	• Expert panel on Healthcare and Public Health Infrastructure data needs for preparedness and response using a Delphi method
MITRE $3.18M	FY06	• CIP, Vulnerability Assessment, and Credentialing
Digital Infusions $350,000	FY06	• Development of an operating capability for the CIDS – merging data fields into automated reports to provide analytical capability for the Secretary's Daily Report during a disaster
RTI $78,000	FY06	• Translating CIDS data to Geographic Information System's (GIS) maps for rapid needs assessment of critical infrastructure
MITRE $1.998M	FY05	• Critical Infrastructure Protection
American Burn Association $210,000 + $349,000 for travel and per diem	FY05	• Advanced Burn Life Support training with Memorandums of Understanding with ABA certified hospitals for clinical training
INCMCE $150,000	FY05	• Mass Casualty Education Research and Competencies
MITRE $1.5M	FY04	• Information Sharing and Analysis Center concept development and Secretary's Operations Center analysis
INCMCE $100,000	FY04	• Curriculum Development for online mass casualty education
ADVISOR		
University of Washington, School of Nursing	FY07	• Environmental health and disaster nursing training
Munroe Regional Medical Center	FY06	• Regional surge capacity for burn, bomb, and blast disasters
University of Tennessee, College of Nursing	FY05	• Homeland security program development
South Carolina Department of Health and Environmental Control	FY05	• South Carolina Nursing Summit for Public Health Emergency Preparedness to address emergency preparedness for nursing

COUNCILS & WORKGROUPS		
Save the Children – Early Childhood Disaster Preparedness	2007	Advisory group for the identification of review of existing resources, identify ways to prepare children for disaster without scaring them. The focus is primarily resiliency and coping with change
Sigma Theta Tau International – Disaster Preparedness Pre-conference	2007	Advisory group for the development of a disaster pre-conference as part ot the 39 Biennial Convention
National Organization of Nurse Practitioner Faculties – MCI Competencies	2007	Identify education guidelines and essential components for preparing advanced practice nurses for mass casualty incidents. Funded by a grant from the NNEPI and building on the INCMCE competencies. Published the *APRN Education for Emergency Preparedness and All Hazards Response: Resources and Suggested Content*. Available at http://www.nonpf.org/NONPF2005/APRNGuidelinesComplete0707.pdf
American Nurses Association Expert Panel	2006 - 2007	Develop policy statement on standards of care during disaster to be presented at the ANA conference on Nursing Care in Life, Death, and Disaster
National Health IT Policy Council	2006 - 2007	HHS internal policy decisions related to health IT
Interagency Health Information Technology Policy Council	2006 - 2007	The mission is to coordinate federal health IT policy decisions across Federal Departments and Entities that will drive federal action necessary to realize the President's goals of widespread health IT adoption
Information Sharing Council	2006 - 2007	Established by Executive Order 13388 focusing on the Congressionally mandated information sharing environment
National Nurse Emergency Preparedness Initiative – Policy Summit	2006	National forum for the exploration of policy issues related to nurse emergency preparedness. Funded by the Department of Homeland Security, Office of Domestic Preparedness and led by The George Washington University in collaboration with George Mason University.
National Infrastructure Protection Federal Senior Leadership Council	2005 - 2006	Status of the national infrastructure protection plan
National Infrastructure Protection Plan Policy and Planning	2005 - 2006	The goal is to consider and advance NIPP Senior Leadership Council matters of policy and planning that require development, coordination, and discussion by the Sector membership and other supporting agencies or entities, as appropriate, in furthering NIPP

Workgroup		
Critical Infrastructure Protection Policy Coordinating Committee	2005 - 2006	White House policy committee to address Critical Infrastructure Protection
HHS Bioterrorism Council	2003 - 2005	Strategic planning for public health emergency preparedness across HHS
Federal Nursing Service Council	2003 - 2006	Council representing the Chief Nurse Officers of the Army, Navy, Air Force, Reserves, USPHS, American Red Cross, and Veterans' Affairs – served as the Deputy to the Chief Nurse of the USPHS